The association between metabolic health status and smell perception in obesity: behavioral and brain anatomical correlates

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LIST OF ABBREVIATIONS

AG	Acylated ghrelin
BMI	Body mass index
CNS	Central nervous system
FTO	Fat mass and obesity-associate gene
GHSR	Growth hormone secretagogue receptor
HOMA-IR	Homeostatic model assessment of insulin resistance
ODT	Odor detection threshold
PEA	Phenyl ethyl alcohol
SD	Standard Deviation
TG	Total ghrelin
UAG	Unacylated ghrelin
WHR	Waist-hip ratio
WHO	World health organization

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I. INTRODUCTION

In recent decades, obesity has become a major health problem. In Germany, the prevalence is currently around 25%. Since obesity is a major risk factor for many noncommunicable diseases, this serious problem urgently requires innovative prevention and treatment measures. Therefore, in this thesis, I will introduce a sense that has not been sufficiently explored in obesity research to date but holds promise for better understanding and potentially combating obesity: the sense of smell. It is directly linked to brain regions involved in homeostatic regulation of eating and decision making, for example for specific foods (Doty, 2015). Thus, it stands to reason that the olfactory sense plays an important role in eating behavior and diseases related to unhealthy diets, such as obesity. Strikingly, odor perception has been shown to influence diet in general: food odors trigger food cravings and appetite and thereby influence for example food choice and meal size selection (Gaillet-Torrent et al., 2014). Beyond that, individuals with hyposmia, i.e. reduced smell ability, prefer high-fat and high-sugar foods compared to individuals with normal sense of smell (=normosmia) (Duffy et al., 1995; Manesse et al., 2017). Obesity, which is associated with altered eating behavior, has recently been linked to impaired olfactory function (Peng et al., 2019). In conclusion, the olfactory system may be a promising target for influencing eating behavior and the control of body weight.

1. The obesity pandemic

Worldwide 39 % of the population are overweight and additional 13 % obese (WHO, 2018). As obesity is preventable and it has dramatically increased in recent decades (Figure 1), it is imperative to understand its causes and consequences. Obesity is characterized by excessive accumulation of body fat with a body mass index (BMI) greater than 30 kg/m². The BMI is defined as a person's weight in kilograms divided by the square of their body height in meters (kg/m²). It is the most used measure to classify weight status into normal weight (18.4 – 24.9 kg / m^2), overweight (25 – 29.9 kg / m^2), class I obesity (30 – 34.9 kg / m^2), class II obesity (35 – 39.9 kg / m^2), class III obesity (BMI > 40 kg / m^2)(WHO, 2018). Nonetheless, BMI must be interpreted with caution because it does not measure the fat mass of the body, but only takes a person's weight and height into account (Nuttall, 2015). Thus, even people with high muscle mass can be classified as obese, even though they do not have the potentially dangerous abdominal fat that is localized around the organs and is the main cause of obesity-related diseases (Lapidus et al., 1984; Ohlson et al., 1985; Fox et al., 2009).

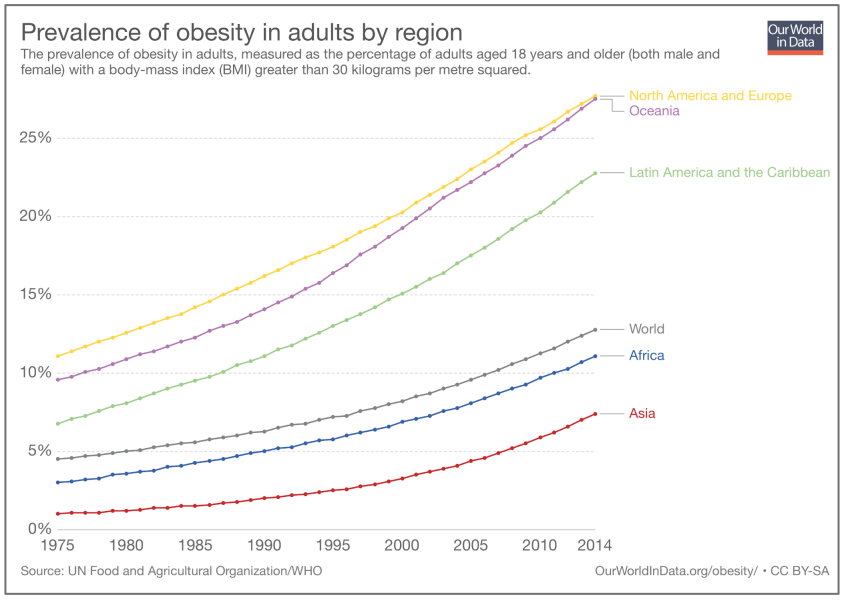

Figure 1: Prevalence of obesity between 1975 – 2014. (reprinted with permission from 'Our World in Data', Ritchie & Roser, 2017)

Especially the amount of potentially dangerous fat accumulations should be considered. Therefore, an additional measure has been introduced which reflects potential abdominal and visceral fat: waist-to-hip ratio (WHR). It is easy assessable and defined as the ratio of the circumference of the waist to that of the hip. The WHO recommends using the WHR as an additional measure to BMI in order to take the importance of visceral fat accumulation for comorbid diseases in obesity into account. Thereby, abdominal obesity is defined as a WHR of above 0,90 in men and above 0,85 in women (WHO, 2011). Obesity represents a major risk factor for chronic diseases such as cardiovascular diseases, metabolic syndrome, diabetes, stroke, and cancer (Rosenthal et al., 2017). Those noncommunicable diseases are the leading cause for death in Europe and America (WHO, 2011). On a very recent note, obesity increases mortality in coronavirus disease 2019 (Covid-19) (Tartof et al., 2020). Hence, preventing obesity could save lives and contribute to an improvement in quality of life. This obesity pandemic must be tackled by incorporating its highly multifactorial etiology, including behavioral, environmental, socioeconomic, and genetic factors. All those factors result in a positive energy balance caused by overeating and a lack of physical activity.

But why has the prevalence nearly tripled over the last 40 years? Most likely, the interaction of changing environmental, socio-economic and individual living conditions is responsible for the continued rise in obesity (Hruby and Hu, 2015). We nowadays live in an increasingly

obesogenic environment. We are faced with a change in lifestyle towards sedentary work- and leisure activities with a negative impact on metabolic health and body weight (Ortega et al., 2018). Car and urban transport infrastructure has increased, supporting physical inactivity and thereby promoting weight gain (Litman, 2013). In western countries foods and especially highly processed foods are available at low cost and any time (Llewellyn, 2018). Additionally, we are constantly influenced by visual advertising designed to entice us to buy and eat something. While most of the advertisement traditionally encompasses visual stimuli, a recent strategy targets the sense of smell: amplification of smells are used to lure us to fast food restaurants, bakeries, etc. (Krishna, 2012; Spence, 2015; Belfort-DeAguiar and Seo, 2018).

In sum, the rate of obesity has increased in recent decades due to changing lifestyle and higher food availability. Since obesity is associated with severe health consequences it is essential to further understand the underlying mechanisms to combat the obese pandemic.

2. Hormones involved in obesity and olfaction

Hormones play indubitably a major role in regulating food intake and physical activity by exerting influence on the energy homeostasis system. In a complex interplay of peripheral and central processes, this system signals the acute hunger state (= being hungry or sated), but also long-term information such as the state of adipose store (for review see: Murphy and Bloom, 2006). Interestingly, many hormones involved in homeostatic circuits also influence olfactory behavior by modulating olfactory sensitivity (Tong et al., 2011a). In this thesis, I will elucidate the interaction between olfaction and homeostatic signaling in obesity.

2.1 Hormones in the regulation of eating behavior and obesity

Being hungry or sated is accompanied by specific hormonal patterns. In healthy humans, levels of orexigenic hormones (= inducing hunger) are increased in the hungry state and they decrease after meal intake (Müller et al., 2015). These hormones have stimulatory effects on eating and are hence associated with hunger and appetite. They include ghrelin (acts peripherally), neuropeptide Y, endocannabinoids and galanin (all three act centrally). Hormones involved in satiety circuits, on the other hand, are referred to as anorexigenic hormones (= satiety-inducing). They exert appetite-inhibiting effects and are consequently associated with meal termination. These inhibitory hormones rise after food intake (Korek et al., 2013). Known representatives are for example insulin, cholecystokinin, leptin, glucagon-like peptide 1 and bombesins. In this thesis, I will focus on insulin and leptin as anorexigenic signals and ghrelin as an orexigenic signal in energy homeostasis. They are all peptide hormones involved in maintaining short- and long-term energy balance and are known as so called ‘adiposity signals’ (Cummings, 2006), i.e. hormones related to body fatness.

On the peripheral level, hormones regulate blood glucose and adipocyte function (Drucker, 2007; Cohen and Spiegelman, 2016). They interact to stimulate or suppress the release and action of other hormones. For instance, ghrelin suppresses the inhibiting effect of insulin on neuron activation in the vagal afferent nerves and thereby informs the brain that the current metabolic state (insulin-dominant vs. ghrelin-dominant) of the body has changed (Iwasaki et al., 2015). Hormones that are involved in eating behavior can activate central appetite circuits in the hypothalamus and brainstem, which are the responsible brain regions for the regulation of appetite and energy homeostasis (Schneeberger et al., 2014). Strikingly, obesity is accompanied by several endocrine and metabolic shifts that are involved in homeostatic

signaling. On the one hand, several hormones with anorexigenic effects are upregulated (Schwartz et al., 1996; Kim et al., 2017). On the other hand, several hormones with orexigenic actions are downregulated in obesity. The mechanisms behind these at first sight counterintuitive alterations are not fully understood yet. However, it has been observed that peripheral and central sensitivity to anorexigenic hormones is lower in obesity. It is therefore possible that a higher amount of these hormones is produced as a compensatory mechanism for hormone insensitivity (Czech, 2017). Alternatively, it is as well plausible that higher levels of these hormones lead to resistances (Erion & Corkey, 2017). In the following section, I will introduce the three hormones on which this thesis focuses in detail and discuss their action:
Insulin is a protein, which is mainly synthesized by the pancreatic beta cells and is produced in response to increased circulating glucose levels, i.e. normally in response to food intake. Insulin acts mainly in the short-term regulation of satiety, i.e. insulin levels respond quickly after food intake, with a peak in insulin concentration approximately 60 min after meal intake (Verdich et al., 2001). The release of insulin stimulates the uptake of glucose in various organs and exerts its satiating effect mainly at the level of the hypothalamus in the brain (Williams et al., 2011). In obesity, circulating plasma insulin levels have been shown to be elevated, while cells appear to be resistant to insulin (Sims et al., 1973). This reduces insulin transport to the central nervous system (CNS) (Baskin et al., 1985). The mechanism underlying insulin resistance is not yet fully understood. However, it is associated with inflammatory processes caused by excess abdominal fat (Chen et al., 2015) and, in particular, with an intracellular increase in triglycerides and fatty acid metabolites (Shulman, 2000). Insulin resistance is a major component of the metabolic syndrome and an early symptom of type 2 diabetes. In the publications included in this thesis, insulin resistance is calculated using circulating fasting glucose and insulin levels with the ‘homeostatic model assessment’ (HOMA-IR) (Gutch et al., 2015).
Leptin is another important anorexigenic hormone. In contrast to insulin, in addition to short-term signaling, it is more involved in long-term signaling of saturation. In healthy individuals, leptin is a satiety signal that regulates energy homeostasis. It is associated with losing weight and improving glycemic control when administered (Heymsfield et al., 1999). This hormone is a product of the human obese gene, firstly described in 1994 (Zhang et al., 1994) and is mainly produced by adipose tissue, i.e. peripheral adipocytes. Thus, circulating levels increase proportionally to body fat percentage and are therefore frequently upregulated in obesity (Schwartz et al., 1996). In rare cases, obesity is associated with leptin deficiencies, that can be balanced by medication (Farooqi et al., 2007). Beyond that, obesity is accompanied by leptin

resistance caused by impaired signaling of the leptin receptor (Lubis et al., 2008) and decreased leptin transport to the CNS (Caro et al., 1996).

Ghrelin is an important hormone with orexigenic function: a peptide hormone, that is mainly produced in the stomach. It is responsible for stimulating meal intake, fat deposition and growth hormone release (Kojima et al., 1999; Wren et al., 2001). Beyond these well-known functions, it is involved in glucose and energy homeostasis, cardiac functions, bone metabolism and cancer development (Pradhan et al., 2013; Müller et al., 2015). Ghrelin is the ligand of the growth hormone secretagogue receptor 1a (GHSR1a), through which it exerts its peripheral and central influences (Kojima et al., 1999). It increases in the fasted state when the stomach is empty and induces feelings of hunger and appetite (Tschöp et al., 2000). Since ghrelin is a fast acting hormone, it decreases quickly after food intake with a dip approximately 60 min after meal onset (Sun et al., 2016). Postprandial decrease in ghrelin is proportional to the amount of food intake in healthy individuals with normal weight (Callahan et al., 2004). Ghrelin circulates in the blood serum in mainly two forms: the acylated (AG) and unacylated (UAG) ghrelin. The acylation is catalyzed by the enzyme ghrelin O-acyltransferase (Yang et al., 2008). AG and UAG act partly together but also have separate functions in the ghrelin system (Delhanty et al., 2012; Heppner et al., 2014). In the context of energy homeostasis, AG regulates glucose homeostasis (Stark et al., 2015), inhibits insulin secretion (Park et al., 2012) and regulates glucagon secretion (Chuang et al., 2011). Thereby it acts in the CNS to induce appetite, and stimulate food intake (Nakazato et al., 2001). UAG on the other hand is the functional inhibitor of AG. By suppressing total ghrelin levels, glycemic control is improved by UAG (Broglio et al., 2004; Delhanty et al., 2012; Heppner et al., 2014). Surprisingly, circulating ghrelin levels are reduced in obesity, although ghrelin has an appetite-stimulating effect. (Tschöp et al., 2001; Oner-Iyidoğan et al., 2007; Pacifico et al., 2009a; Dardzinska et al., 2014). Tschöp et al (2001) suggested that this may be due to a physiological adaptation to the positive energy balance that prevails in the obese state. It is also conceivable that genetic influences, such as modification of GHSR or obesity-associated FTO genes, are involved in the altered function of ghrelin (Solomou and Korbonits, 2014). A striking feature of ghrelin regulation in obesity is an elevated ratio between AG and UAG. The ratio plays a crucial role in maintaining weight balance and is discussed to be a major player in the development and maintenance of obesity (Barazzoni et al., 2007; St-Pierre et al., 2007; Pacifico et al., 2009b). Another striking feature of ghrelin regulation in obesity is reduced ghrelin reactivity after food intake: there is only an attenuated reduction in total ghrelin levels after eating (le Roux et al., 2005). In 2014, this could also be studied for the different forms of ghrelin: AG levels were

shown to decrease postprandially in normal-weight individuals, whereas they did not change after food intake in individuals with obesity (Dardzinska et al., 2014). Due to the appetite-stimulating function of AG, this could mean that people with obesity might experience a constant high feeling of hunger and appetite even after food intake (Dardzinska et al., 2014).

2.2 Hormones in the context of smell perception

Palouzier-Paulignan et al. introduced a neurophysiological model describing the neuroanatomical and -physiological link between the endocrine and olfactory systems (2012). They emphasize the role of the olfactory system as an internal sensor of nutritional status. The existence of receptors for hormones that are involved in homeostatic signaling in peripheral and central olfactory regions underpins this idea. Further support for the proposed link between both systems is that olfactory perception adapts in response to meal intake with its consequently changing levels of hunger hormones (Stafford and Welbeck, 2011; Ramaekers et al., 2016). Interestingly, it has been shown that intranasally applied insulin in humans with normal weight directly decreases olfactory sensitivity (Brunner et al., 2013). Since insulin levels normally rise in response to meal intake, lowered odor sensitivity would be beneficial to terminate meal intake and inhibit further eating. A similar effect has been found for leptin in rats: leptin administration led to decreased sniffing behavior and decreased locomotor activity (Prud'homme et al., 2009) as well as decreased olfactory sensitivity (Julliard et al., 2007). This might lead to reduced search for food sources. Further, it has been shown that intravenously administered ghrelin in animals and humans, increases olfactory sensitivity (Tong et al., 2011b). Since heightened ghrelin levels mimic hunger, increased olfactory sensitivity may be beneficial in identifying potential food sources in the environment (Tong et al., 2011b). Strikingly, peripheral, and central olfactory regions have a high density of hormone receptors that are involved in short and long-term homeostatic signaling. For instance, leptin receptors are expressed in the olfactory mucosa and olfactory bulb, and to a lesser extent, leptin is also synthesized in the olfactory mucosa (Baly et al., 2007). Last but not least, the olfactory bulb is the brain region with the highest density of insulin receptors (Hill et al., 1986) as well as highest insulin concentration (Baskin et al., 1983). More specifically, it is currently under debate whether the insulin found in the olfactory mucosa and olfactory bulb plays an active role in energy homeostasis by regulating olfactory perception (Lacroix et al., 2008).

In summary, it can be stated that olfactory and endocrine systems are strongly intertwined. However, it is poorly understood whether and how obesity related hormonal changes are

associated with olfactory processing in humans. Open questions in that regard are (i) whether olfactory function adapts differently to internal status in obese and normal weight people and (ii) whether obesity related hormonal changes are associated with olfactory function and alterations in olfactory regions of the brain. Answering these questions would contribute to a deeper understanding of hormonal mechanisms that might underlie decreased olfactory function in obesity.

3. The olfactory system

Being the phylogenetically oldest sense, the importance of the olfactory system in humans is largely underestimated. However, smells indeed play an important role in diet, in mate choice and in human safety as an olfactory warning signal, for example, of rotten food (Doty, 2015). The sense of smell involves a complex system that integrates external signals from the surrounding through the nose and oral cavity, as well as receiving internal signals from the brain and body, which in turn influence odor perception (Julliard et al., 2017). These perceptions guide our eating behavior (Wedekind et al., 1995; Stevenson, 2010; Boesveldt and de Graaf, 2017), e.g. by detecting potential food sources in the environment, selecting suitable and wanted foods, as well as hormonally anticipating and preparing the body for a meal (Boesveldt and de Graaf, 2017; Proserpio et al., 2017). Beyond that, it is widely recognized that olfaction contributes essentially to flavor perception of foods and beverages (Spence, 2015) and can significantly influence food preferences.

3.1 Anatomy and physiology

Humans perceive odors from the environment through the nose (=orthonasal olfaction) or through the mouth, more specifically through the oral cavity, while chewing (=retronasal olfaction) (see Figure 2). Thereby odor molecules are either inhaled via the nostrils or odor molecules move up the oral cavity to the nasal cavity.

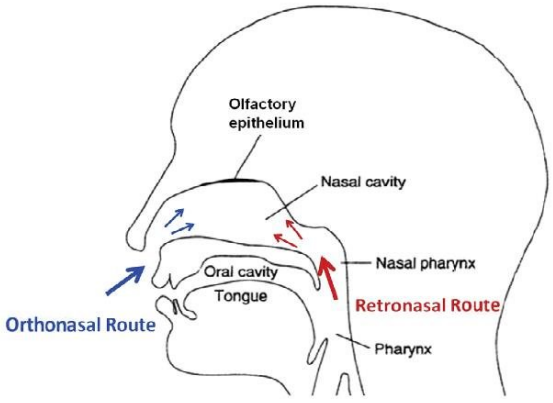

Figure 2: Orthonasal and retronasal routes of olfactory perception, source: Goldstein et al. (2010)

To illustrate the processing of odors, the anatomy of the olfactory system is explained in more detail (see Figure 3): the nose is a bony and cartilaginous structure, consisting of two nasal passages that are subdivided by the nasal septum.

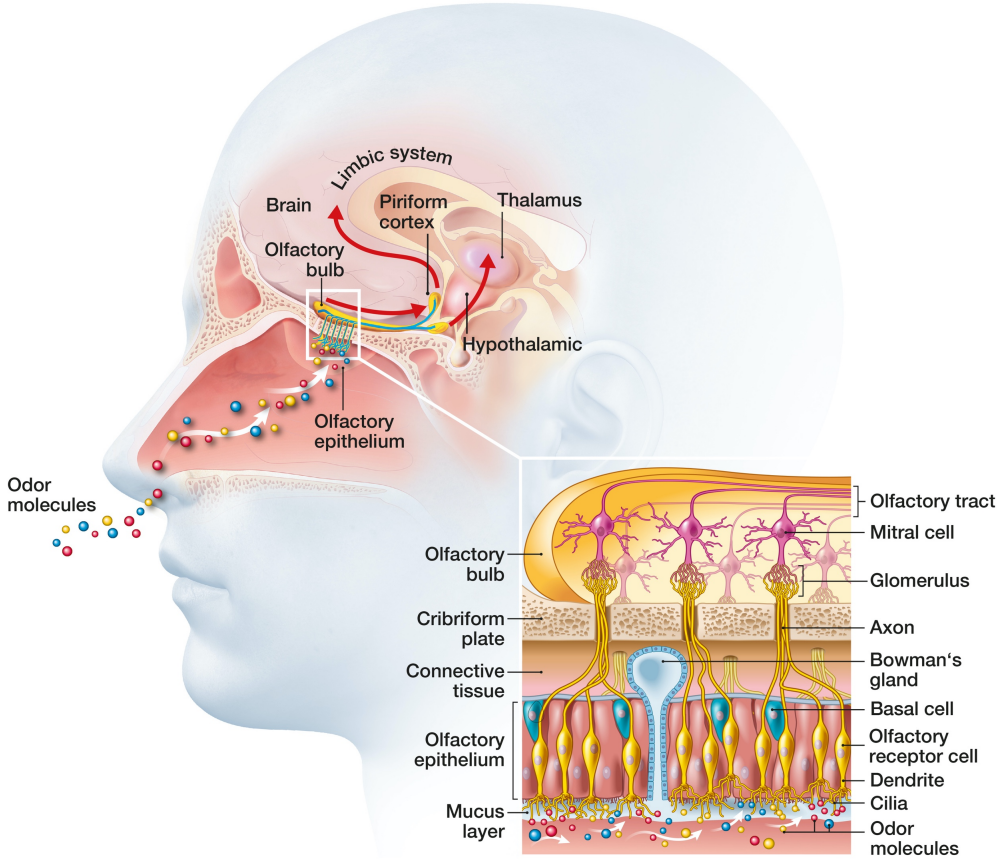

Figure 3: Schematic representation of odor molecules entering the nasal cavity and the pathways of odor processing in the brain. (Image acquired from AdobeStock, illustrator: Axel Kock)

Inside the nose, those structures are covered with the mucus-secreting olfactory epithelia. Important for the perception of odors is sufficient air circulation for the maintenance of moisture in the nose. Therefore, in the nasal chambers on both sides are the inferior, middle, and superior turbinates, which are shell-shaped spongy bones that increase the volume of the nose, allowing air to circulate. Further up in the nasal cavity are the olfactory clefts within the upper turbinates. Here the olfactory neuroepithelium with olfactory receptors is located, which is particularly important for the transmission of olfactory information to the brain. Crucially, the olfactory receptor proteins are members of a large g-protein receptor family and consist of

approximately 1000 genes (Zozulya et al., 2001). They hence comprise the largest multigene family in the mammalian genome and are expressed in the membranes of olfactory receptor neurons that are responsible for odor detection in the surroundings. The partition between nasal cavity and brain is called the cribriform plate, a sponge like bony structure. It is pierced from the nasal cavity and the brain respectively with olfactory nerves offering a direct route to the brain. The first entered brain region in that circuit are the olfactory bulbs. It is a paired ovoid-shaped brain structure, responsible for filtering and modifying sensory input through the nose. The olfactory bulbs receive many fibers from the primary olfactory cortex and central structures within the brain (Gottfried, 2006). The major brain regions that are involved in olfactory processing include the piriform cortex, the orbitofrontal cortex, and the hypothalamus.[1] While the pirifrom cortex is responsible for signaling the intensity of odors (Rolls et al., 2003), the orbitofrontal cortex conveys information about the pleasantness and reward value of an odor (O'Doherty et al., 2000; Rolls, 2015). The hypothalamus is involved in reward processing of odors and tastes in response to internal signals of hunger and satiety (Rolls, 2014). To summarize the circuit of odor processing: odor molecules enter the olfactory mucosa through the oral and nasal cavity and bind to olfactory receptors. From there, the information is transferred through the olfactory nerves that lie within the cribriform plate to the olfactory bulbs and from there to higher order brain regions.

Olfactory bulb volume

An essential region in the olfactory system is the olfactory bulb (depicted in Figure 4). Here olfactory information is firstly processed and passed on to higher order olfactory brain regions. Importantly, the size of the olfactory bulbs is positively correlated with olfactory function in health (Buschhüter et al., 2008; Mazal et al., 2016) and disease (Liu et al., 2017; Negoias et al., 2010; Turetsky et al., 2000). Several mechanisms for this phenomenon come into play: on the one hand insufficient afferent input from the olfactory epithelium and olfactory receptor neurons to the olfactory bulbs might cause a reduction in olfactory bulb volume (Gudziol et al., 2009). On the other hand, it might as well be plausible that centrally controlled neurodegenerative processes cause a reduction in the volume of the olfactory bulb. This has been demonstrated particularly in neurodegenerative diseases such as Alzheimer's and Parkinson's disease (Attems et al., 2014). In obesity, it is unclear whether low olfactory

[1] The topic of neural processing of odors will be addressed in more detail in the dissertation of Nora Breuer (expected to be submitted in 2022).

function is reflected in neuroanatomical changes in the olfactory bulb. Intriguingly, they have a high density of hormonal receptors involved in homeostatic signaling, such as receptors for insulin and leptin (Baskin et al., 1983; Thanarajah et al., 2019; Havrankova et al., 1981; Marks et al., 1990). Therefore, one of the central hypotheses of this thesis is to investigate the possible influence of metabolic health markers, particularly insulin resistance, on potential changes in the volume of the olfactory bulb in obesity.

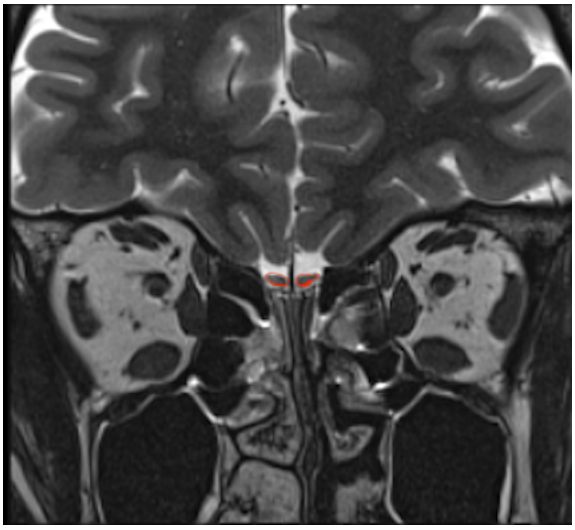

Figure 4: Position of the olfactory bulb in the human brain (healthy control subject from study 3 (26 years old, female). Olfactory bulbs are delineated in red.

Factors influencing olfactory perception

Due to its exposed position with direct access for potential pathogenic agents and other external influences, the olfactory system is very susceptible to irritations and malfunction (Witek, 1993; Dando et al., 2014). For instance, physical factors such as cold weather decrease olfactory function and high humidity improves it (Kuehn et al., 2008; Riveron et al., 2009; Martin et al., 2011) and even small respiratory infections negatively influence olfactory performance (Pellegrino et al., 2017). Smoking also appears to have a major impact on smell perception: olfactory function is impaired and recovers only slowly after quitting (Hayes and Jinks, 2012). In the context of this work, it is interesting to note that smokers who have only been smoking for a short time still show normal olfactory function, but already have reduced OB volume (Schriever et al., 2013). This might indicate that the impairment of olfactory function is already reflected in brain anatomy before a noticeable smell impairment occurs. Beyond external factors, olfaction is also modulated by long- and short-term hormonal changes within the body,

as the olfactory and hormonal systems are closely linked (Doty and Cameron, 2009; Lacroix et al., 2015). On the one hand, this is important in the context of energy homeostasis (as discussed in more detail in section 2.2), since olfactory perception depends on hormonal changes associated with homeostatic signaling, e.g. ghrelin enhances olfactory performance (Palouzier-Paulignan et al., 2012). On the other hand, this is also important in the context of sex hormones: women exhibit higher olfactory performance than men (Doty and Cameron, 2009; Ohla and Lundstrom, 2013). The underlying causes of differences in odor perception and processing might be primarily due to hormonal- and physiological differences between males and females (Oliveira-Pinto et al., 2014, Doty and Cameron, 2009), but there is also evidence that social and emotional differences play a role (Ohla and Lundstrom, 2013; Boesveldt et al., 2017). In women odor perception additionally changes over the menstrual cycle: sensitivity to odors increases in the follicular phase and decreases in the luteal phase (Derntl et al., 2013; McNeil et al., 2013). Since humans unconsciously rely on their sense of smell in mate choice (Wedekind et al., 1995), higher olfactory performance in follicular phase (ovulation takes place) is highly reasonable to find a suitable mate.
To conclude, these factors make the interpretation of olfactory function very difficult and prone to error due to the many internal and external influencing variables.

3.2 Measuring smell ability: three dimensions of olfactory function

There are several psychophysical tests to quantify olfactory function in the clinical and the research setting (for an overview see Eibenstein et al., 2005; Doty, 2015). In Europe, the Sniffin' Sticks test battery (Burghart®, Wedel) developed by Kobal und Hummel (1996) is widely used to assess olfactory function. It is a well validated and standardized instrument and covers three aspects of olfactory performance: odor identification, odor threshold and odor discrimination ability (Hummel, Sekinger, Wolf, Pauli, & Kobal, 1997). Odor identification and odor discrimination tests are associated with cognitive function, especially memory, while the olfactory threshold test reflects sensitivity to odors and accordingly maps peripheral olfactory function (Hummel et al., 2007). The Sniffin’ Sticks test battery consists of odorized pens, which are filled with a tampon that carries the odorant. The pens are presented by the experimenter, who removes the cap and swivels the pen approximately 1-2 cm under both nostrils for 3 seconds. The duration of the whole test battery is around 30-45 minutes (Wolfensberger, 2000). To avoid sensory adaptation to the odorants, consecutive trials are usually separated by 30 sec breaks, which results in a total trial length of at least 45-48 sec

according to Rumeau et al. (2016). Moreover, a break of 3-5 min between subtests must be maintained. The application of the threshold subtest, however, is highly variable in duration (10 – 25 min) and rather time consuming, rendering it demanding for patients and study participants in terms of perceptual and attentional resources. Thus, shortened and less variable overall test duration would be beneficial in clinical and research routine to use patient time efficiently, allowing for complex study designs, and minimizing the patient's / participant's workload.

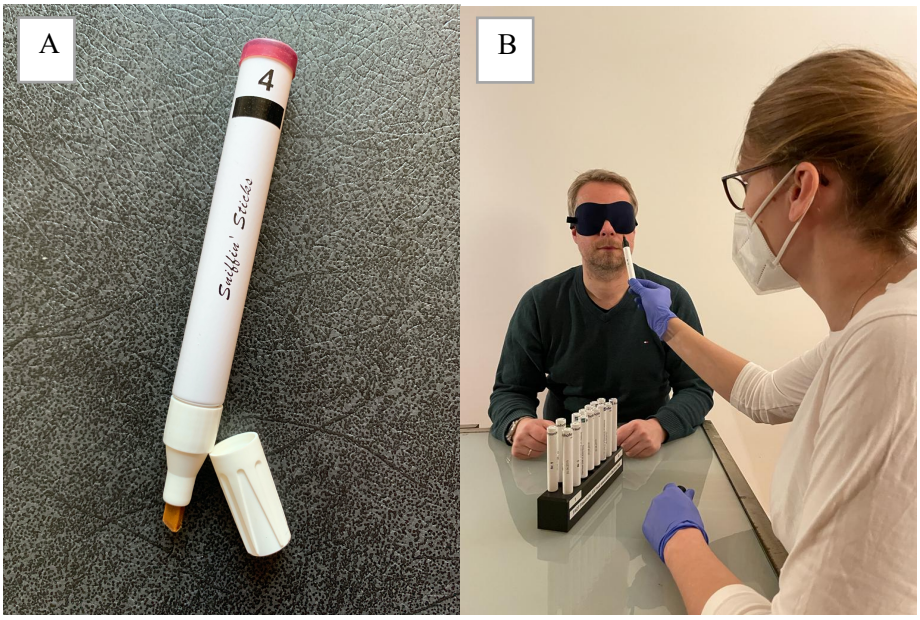

Figure 5: A - Sniffin' Sticks odorized pen with felt tip (pen opened) B - Administration of Sniffin' Sticks.

3.3 The role of olfaction in the control of eating behavior

The influence of olfactory perception on our eating behavior in everyday life can be explained by means of an example: imagine you are walking through the city center and suddenly you smell pizza somewhere. You are not hungry, so you try to keep walking, but somehow the smell magically draws you in. Without thinking about it, you suddenly buy a slice of pizza and eat it. As shown here, the sense of smell plays a crucial but underestimated role in eating behavior: smells signal the availability and identity of potential foods in our environment (Rolls, 2007). And due to the direct pathway of odor signaling into reward-related brain areas, smells have the power to trigger the desire to eat (Jansen et al., 2003).

Generally, the regulation of eating behavior underlies the influences of current homeostatic needs and the hedonic value of certain foods (Saper et al., 2002), which are both intimately linked to smell perception. In animals it has been shown for example that smell perception adapts in response to internal needs: sensitivity to odors increases in the hungry and decreases in the sated state (Aime et al., 2007). In humans, however, results are divergent and show both higher (Hanci and Altun, 2015a) and lower sensitivity (Albrecht et al., 2009) in fasted states, or no difference at all (Enck et al., 2014). Regarding the hedonic value of foods, odors also seem to play an important role here. They are particularly associated with food cravings. Thereby external olfactory cues can stimulate appetite and trigger craving for a certain food (Firmin et al., 2016). Odors elicit physical reactions such as increased saliva production and the release of hunger-relevant hormones, which in turn prepare the body for the expected calorie intake (Proserpio et al., 2017). These sensations and physiological reactions influence the decision whether, what and how much we eat. In this respect, it has recently been shown that implicit stimulation with food odors has an influence on food choice and portion size (Chambaron et al., 2015): for example stimulation with a fruity odor made people prefer a fruity desert to a chocolatey desert (Gaillet-Torrent et al., 2014).

3.4 Smell perception in obesity

Our environment is full of sweet beverages and high-energy foods advertised through various sensory channels. Strikingly, individuals with obesity are more susceptible to those external food cues, such as smells (Proserpio et al., 2019). Since it has been shown that environmental odors influence food choice (Stroebele and De Castro, 2004; Gaillet-Torrent et al., 2014), they might play an important role in unvoluntary eating that occurs with obesity.

Previous studies suggest that smell perception is altered in obese individuals. While they perceive food odors as more pleasant than lean individuals (Stafford and Whittle, 2015), the overall olfactory function is low (Peng et al., 2019). The mechanisms behind these alterations have been scarcely investigated yet. Low smell capacity in adult human obesity has been shown for the first time in 2004: in an odor identification test individuals with morbid obesity (BMI > 40) performed worse than individuals with obesity with a BMI < 40 (Richardson et al., 2004). Several subsequent studies confirmed that individuals with obesity might suffer from low smell ability (Simchen et al., 2006; Patel et al., 2015b; Skrandies and Zschieschang, 2015; Fernández-Aranda et al., 2016; Pastor et al., 2016). But why might olfactory function be impaired in obesity? Current explanatory models include first and foremost metabolic and

endocrine alterations in obesity (Richardson et al., 2004; Peng et al., 2019). Strikingly, many hormones that have altered function in obesity are very important players in olfactory function. For example, intranasally applied insulin decreases olfactory function (Brunner et al., 2013). In obesity, insulin levels are usually elevated and therefore could be related to decreased olfactory function. Another example is the hormone ghrelin. It is usually positively correlated with olfactory performance (Fernández-Aranda et al., 2016), i.e. low ghrelin levels are associated with low olfactory function. Since ghrelin levels are lower in obesity when compared to individuals with normal weight, this factor might contribute to impaired smell perception. A study from Fernandez-Garcia et al. (2017) supports the notion that metabolic factors might be crucial for changes in olfactory function: they showed that low olfactory ability is better predicted by visceral fat mass that is accompanied by inflammatory processes than by BMI. Other factors influencing olfactory function in obesity might be genetics: Zamora et al. (2012) showed that olfactory processing speed is associated with genetic alterations in obesity. Hence, genetic influences could also play a role in understanding impaired olfaction in obesity. This notion is supported by animal studies: it has been shown that diet-induced and genetic-induced obese mice differ in their olfactory performance regarding detecting scented foods, memorizing odors correctly and discriminating them (Tucker et al., 2012). Further, external factors such as diet might play a role as well: it has been shown in animals that a hyperlipidemic diet and subsequent obesity is associated with loss of olfactory sensory neurons and consequently a decrease in olfactory function (Thiebaud et al, 2014).

Beside smell performance, also other aspects of smell perception are altered in obesity. For instance, individuals with obesity are more likely to form vivid mental images of food and associated food odors (Patel et al., 2015a). This plays a crucial role in food cue reactivity and possible food consumption. As food cue reactivity to visual food stimuli is heightened in individuals with overweight compared to those of normal weight, reactivity to food odors might also be higher in individuals with overweight or obesity (Tetley et al., 2009). Additionally, it has been shown that food preference in individuals with overweight compared to those of normal weight is less affected by internal state (Zoon et al., 2014). This could support the idea of higher reactivity to external rather than internal signals, what might in turn lead to overeating and choosing high-caloric foods without physiological needs.

Current shortcomings of recent studies on smell perception in obesity

One important shortcoming of olfactory studies in obesity is that except for one study only non-food odors have been used to measure olfactory function. Most studies investigated odor

sensitivity using the standard non-food odors from the Sniffin' Sticks test battery: n-butanol (fermentation-like) or PEA (rose-like). Strikingly, the one study that applied a food odor showed that obese (BMI > 30) individuals performed even better than non-obese (BMI < 30) in an odor threshold test for a sweet, high-caloric food odor (chocolate) (Stafford and Whittle, 2015). Although this is a very interesting approach, this study has a methodological shortcoming: they assessed olfactory sensitivity with squeeze and sniff bottles, because there is no commercially available threshold test for chocolate. Nevertheless, this result is very intriguing and challenges the hypothesis of a general odor impairment in obesity. Accordingly, smell capacity might not be quantitatively impaired, but qualitative alterations could also be plausible. Besides the proposed idea, another alternative explanation for divergent findings might come into consideration: the degree of obesity. As shown in Table 1 most studies examined the olfactory performance of mainly morbid obesity (class 3 obesity, BMI > 40 kg/m^2) or compared mildly obese to underweight participants. Only three studies investigated participants with mild and moderate obesity with a BMI < 40 kg/m^2 and compared them to healthy controls. Two of the studies showed impaired smell performance (Fernandez-Garcia et al., 2017), but most probably using the same pool of participants, and one enhanced smell performance (Stafford and Whittle, 2015) in subjects with higher BMI, respectively. Generally, the degree of obesity is associated with metabolic health status, including stages of pre-diabetes and circulating levels of hormones that are involved in homeostatic signaling (Slagter et al., 2017). The metabolic health status might have an impact on the functioning of the olfactory system, e.g. on olfactory receptor signaling. This idea is also supported by studies that have shown that olfactory perception is impaired in diabetes (Gouveri et al., 2014) and by the observation that olfactory function improves after bariatric surgery (Hanci et al., 2015b; Holinski et al., 2015).

Table 1: Overview of studies on odor sensitivity in obesity.

Study	**Normal weight group**			**Obese group**			**Finding**	**Comment**
	N	Age in years ± SD	BMI in kg/m^2 ± SD	n	Age in years ± SD	BMI in kg/m^2± SD		
Fernandez-Garcia (2017)	77	27.1 ±7.3	21.6 ±1.7	28	46.4 ±12.2	35.2 ±2.6	Lower ODT (n-butanol)	
Fernandez-Aranda (2016)	36	37.3 ±5.9	22.4 ±2.6	59	37.5 ±8.7	42.7 ±6.6	Lower ODT (n-butanol)	
Pastor (2016)	70	27.4 ±7.36	21.7 ±1.62	26	47.3 ±11.1	35.5 ±2.53	Lower ODT (n-butanol)	
Stafford (2015)	20	19.4 ±.3	20.3	20	20.4 ±.4	31.3 ±.3	Higher ODT (chocolate)	
Skrandies (2015)				7	35.5	34.4	Lower ODT in higher BMI participants	Compared to underweight & normal weight

SD – Standard Deviation; ODT – Odor detection threshold (=olfactory sensitivity)

4. The link: Why target the olfactory system in obesity?

Due to its physiological and anatomical characteristics, our sense of smell offers a unique starting point to change eating behavior. It has a direct connection to the limbic system of the brain (van Hartevelt and Kringelbach, 2012). Thus, olfaction offers direct access to often unconscious choices in eating behavior (Gaillet-Torrent et al., 2014) and offers the opportunity of influence toward a well-balanced and healthy diet.

Of special interest within this thesis are hormones that influence olfaction. They can directly enter the brain via the olfactory mucosa, thereby easily overcoming the blood-brain barrier (Pardeshi and Belgamwar, 2013; Maejima et al., 2015; Patel and Patel, 2017; Schmid et al., 2018). This is especially important because homeostatic hormones have a great impact on eating behavior. Therefore, intranasal administration of hormones may offer an easily accessible option to curb appetite and cravings in the near future.

Strikingly, various receptors for hunger-relevant hormones, such as ghrelin and insulin, are found in the olfactory bulbs (Palouzier-Paulignan et al., 2012). This indicates a possible connection between homeostatic signals (hunger, satiety) and the perception and processing of odors. Conversely, the direct integration of the hypothalamus into the olfactory system allows the modulation of hormone secretion (Wyart et al., 2007). It is discussed whether the close connection between the ventromedial nucleus of the hypothalamus (= involved in energy homeostasis and satiety) and the olfactory bulb could be responsible for a decrease in the hedonic value of an odor. This could be a response to satiation as discussed in more detail by Hirsch (1995). Interestingly, Ulusoy et al. (2016) show that people with better olfactory performance also show a greater decrease in olfactory function when they are full and therefore react less to ambient odors that might induce appetite and tempt to eat. Conversely, congenital anosmic patients exhibit a lower sense of satiety compared to normosmics (Novakova et al., 2012). One might conclude that a healthy sense of smell supports a healthy response to personal physiological needs, while a dysfunctional sense of smell seems to open gates towards uncontrolled eating. In sum, the literature already indicates that smell perception is impaired in obesity. At the same time the literature proposes a relationship between olfaction and hormones that are involved in homeostatic signaling and obesity. However, it is still unclear to what extent hormonal changes in obesity are associated with the altered sense of smell. Therefore, the aim of this thesis is to contribute to a further understanding of mechanisms that might underlie altered odor perception in obesity.

II. RATIONALE OF THE EXPERIMENTAL WORK

The aim of this work was to investigate the mechanisms that might underlie olfactory changes in obesity, especially regarding the internal state (hungry vs. sated) and metabolic health status (HOMA-IR, leptin, TG, AG/UAG level, BMI, WHR). We focused on the olfactory sensitivity to food and non-food odors in direct comparison, which has not been investigated yet. Further, we examined the relationship between metabolic health factors in obesity and neuroanatomical alterations in the olfactory bulbs.

Thus, from the current state of knowledge, we derived the necessity for the following experimental work:

Study 1: To develop a short procedure for assessing odor sensitivity in a complex research design, as previous sensitivity tests are very time and energy consuming for the subjects.

Study 2: To investigate odor sensitivity for food and non-food odors in the hungry and sated state in a balanced sample of participants with normal weight, overweight and obesity.

Study 3: To investigate the association between olfactory bulb size and metabolic health factors in obesity

From the current state of knowledge, we derive the following hypotheses:

(i) Individuals with normal weight (BMI 18.4 – 24.9 kg/m^2) will exhibit higher odor sensitivity for the non-food odors when compared to individuals with obesity (BMI > 30.0 kg/m^2) and vice versa for the food odor.

(ii) Odor sensitivity decreases in the sated when compared to the fasted condition in individuals with normal weight. In obese individuals there is no change in odor sensitivity in response to meal intake.

(iii) The relationship between odor sensitivity and obesity is mediated by metabolic and endocrine health parameters. Specifically, we hypothesize that leptin, HOMA-IR and AG/UAG ratio is negatively associated with smell function and total ghrelin is positively associated with smell function.

(iv) The olfactory bulb volume is lower in obese when compared to normal-weight participants and negatively correlated with HOMA-IR, leptin, and body fat percentage.

III. EXPERIMENTAL WORK

Study 1: Short procedure to assess odor detection thresholds

Rapid assessment of olfactory sensitivity using the "sniffin' sticks"

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Rapid Assessment of Olfactory Sensitivity Using the "Sniffin' Sticks"

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Abstract
Introduction Assessment of olfactory performance is of high clinical interest in the contexts of smell loss as well as neurological diseases, and recently gained attention in obesity research. Available olfactory tests, especially for assessing olfactory sensitivity, are time-consuming and require high cognitive capacity. Therefore, we aimed to establish a short procedure for reliably testing olfactory sensitivity using a subtest of the "Sniffin' Sticks" battery. Evaluation criteria are test duration, validity, and test-retest reliability.
Methods In a preliminary study using a within-subject repeated-measures design, we measured olfactory sensitivity for *n*-butanol in 20 young and healthy participants. We compared sensitivity obtained with three different measures during two sessions in a pseudo-randomized order: a standard single-staircase three-alternative forced-choice procedure with seven reversals (SSP_7); an abbreviated version with five reversals (SSP_5); and an ascending presentation of 16 dilution steps from lowest to highest odor concentration (brief ascending procedure, BAP).
Results Compared to the SSP_7, the BAP was 51%, and the SSP_5 26% shorter in duration. Both the BAP and SSP_5 scores were highly correlated with the SSP_7. The test-retest reliability in all three tests was similar to that typically reported in olfactory research.
Conclusion The abbreviated tests are valid measures of olfactory sensitivity. Especially, the BAP is as reliable as the standard method, but remarkably faster and easier to perform.
Implications Thus, the short procedures bear potential for both research and clinical practice, especially for complex study designs with time constraints on olfactory testing and for patient populations with attention deficits.

Keywords Ascending procedure · Olfaction · Single staircase procedure · Smell · Threshold

Introduction

Olfaction is an integral part of the human sensorium: the recognition of smells from the surroundings is crucial for the detection of hazards such as fire and spoiled foods, but also for social communication and signaling food availability. Measures of olfactory performance have become pivotal in both clinical and research practice. Especially, the olfactory detection threshold (ODT) oftentimes depicts differences between clinical and healthy populations (Krismer et al. 2017; Yazla et al. 2018). It measures the sensitivity to smells, that means, the lowest odor concentration that can reliably be picked up from the environment. ODTs have proven to reveal also small differences in smell perception, for example, between obese and normal-weight populations (Skrandies and Zschieschang 2015). Further, ODT and general olfactory testing is an important tool in the clinical examination of smell loss, as well as the early detection of neurological diseases like Parkinson's and Alzheimer's, as altered olfactory performance presents an early symptom and therefore a possible disease marker. For a review of available olfactory tests, see Doty (2007) and Eibenstein et al. (2005). Here, we focus on the commercially available ODT test kit from the "Sniffin' Sticks" test battery (Burghart, Wedel, Germany), which measures olfactory sensitivity to either *n*-butanol or phenylethyl alcohol. This test kit is well validated in Europe (Hummel et al. 2007), easy to assess by using commercially available pen-like devices, and offers explicit operating instructions

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(Rumeau et al. 2016). However, like most other commonly used ODT tests, the application procedure is rather time-consuming and highly variable, rendering it demanding for patients and study participants in terms of perceptual and attentional resources. The ODT subtest of the "Sniffin' Sticks" has an implementation time of 10–25 min, showing high variability in duration depending on the patient's/participant's concentration capacity and ability to smell. To avoid sensory adaptation to the odorant, consecutive trials are usually separated by 30 s breaks, which results in a total trial length of at least 45–48 s according to Rumeau et al. (2016). Thus, shortened and less variable overall test duration would be beneficial in clinical and research routine to use patient time efficiently, allowing for complex study designs, and minimizing the patient's/participant's workload.

Few investigations tackle the shortening of the ODT subtest of the "Sniffin' Sticks" test battery. To date, the proposed short versions of the ODT test were unable to show acceptable test-retest reliability, stable test duration, and significant time-saving concordantly. Two studies present short versions based on the constant stimuli procedure (CSP) (Fechner 1860), in which the olfactory stimuli are presented once for each odor concentration in a randomized order (Kern et al. 2015; Lotsch et al. 2004). The ODT score is estimated by means of logistic regression (Linschoten et al. 2001). This method is frequently used in psychophysical threshold testing but has two major disadvantages in olfactory testing. Firstly, the interleaved presentation of high and lower odorant concentrations can lead to quick adaptations of the examinee's olfactory system. Secondly, the threshold value is estimated with logistic regression (for further details see (Linschoten et al. 2001)), but to ensure correct classification of the model, several trials for each odor concentration step would be needed. If this were considered, the test duration would be even longer compared to the standard test procedure. Furthermore, Croy et al. (2009) compared a wide step method with only 8 dilution steps to the standard procedure with 16 dilution steps in a healthy and clinical population. They showed an average time-saving of 16–30% depending on the population group (healthy vs. patient) and odor condition (*n*-butanol vs. PEA) when using the wide step method. The test-retest reliability of this method is compared to standard reliability of olfactory testing relatively high (.81–.86) and the test reliably differentiates patient populations from healthy volunteers. However, the wide step method cannot depict subtle differences between groups, since it has only 8 instead of 16 dilution steps of the odor.

Recently, Sijben et al. (2017) proposed an alternative short version using the ascending limits procedure (ALP) as described by Cain et al. (1988). The authors showed that thresholds obtained with the ALP are similar to those obtained with the standard single staircase procedure (SSP); however, compared to the SSP, the ALP shows comparably high variability in duration and an average time-saving of only 5 min.

Here, we evaluate shortened procedures using the "Sniffin' Sticks" ODT test kit that circumvent these limitations. To ensure stable test duration, simplify testing, and avoid sensory adaption, we use the brief ascending procedure (BAP)—an integration of both the previously discussed CSP and ALP—and compare it to the standardly used SSP and a shortened SSP version. Evaluation criteria are test duration, validity and test-retest reliability.

Materials and Methods

Subjects

A total of 20 participants (10 women; mean age 24.68 years, SD 2.6 years, range 19–30 years; mean body mass index (BMI) 22.03 kg/m^2, SD 1.66 kg/m^2, range 19.77–25.07 kg/m^2) took part in the experiment. All participants were previously screened by means of telephone interviews. Exclusion criteria included current smoking, recent history of smoking (< 3 years of abstinence), vegetarian/vegan diet, allergies, current use of medication except oral contraceptives, drug use within the last 2 months, alcoholism, current pregnancy/breastfeeding, any subjective or objective impairments of the sense of smell, nose surgery except childhood nasal polypectomy, and history of neurological or psychiatric disorders. Inclusion criteria were age between 18 and 36 years. After inclusion, participants provided written informed consent. The study was carried out in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the University of Leipzig.

Sample Size Estimation

A priori we determined the minimum number of participants required using a statistical power analysis with Gpower software (Faul et al. 2007). Based on data from Hummel et al. (1997), we performed the sample size estimation. They correlated odor thresholds that were assessed with the "Sniffin' Sticks" test battery, at two different test days. The effect size in this study was $r = .61$, considered to be large using Cohen's (1988) criteria. With an alpha = .05 and power = .80, the projected sample size needed with this effect size (GPower 3.1) is approximately $n = 16$. Thus, our proposed sample size of $n = 20$ will be more than adequate for the main objective of this study.

Study Design and Procedure

The investigation involved ODT testing on two test days in a repeated measures within-subject design. Participants were instructed to refrain from eating and drinking except for water 2 h prior to testing. In the first session, all participants were

screened for olfactory function using the short form of the olfactory identification test included in the "Sniffin' Sticks" test battery (Mueller and Renner 2006). On both test days, olfactory testing was conducted using the single staircase procedure (SSP) as described by Hummel et al. (1997) and the brief ascending procedure (BAP), in a pseudo-randomized order. The interval between test days was approximately 1 week (mean 8.45 days, SD 4.37 days, interval 7–20 days). ODT tests were conducted successively with a short break of approximately 10 min. After conducting both ODT tests, participants rated intensity (0 = very weak, 10 = very strong), pleasantness (− 5 = unpleasant, + 5 = pleasant), and familiarity (0 = unfamiliar, 10 = familiar) of the odor from the pen containing the highest concentration of *n*-butanol on a visual analog scale (Aitken 1969).

Materials

All odorants were presented in commercially available felt-tip pens ("Sniffin' Sticks"; Burghart Instruments, Wedel, Germany). For the screening of olfactory function, we used the short form of the olfactory identification test from the "Sniffin' Sticks" test battery (Mueller and Renner 2006). In a multiple-choice task, participants must identify the correct smell from a card with four descriptors per odorant. In total, five odorants were presented; the test confirms the presence of normosmia (≥ 4 correct answers) or hyposmia (< 4).

The ODT test kit from the "Sniffin' Sticks" test battery is performed with *n*-butanol, an odorant that arises from fermentation processes and is frequently used in olfactory testing. It is perceived as rather unpleasant.

Sixteen dilutions of *n*-butanol are prepared by stepwise diluting previous odor concentrations in a ratio of 1:2. The strongest odor concentration is 4% (pen number 1) and the weakest is 1.22 ppm (pen number 16).

The odorized pens are presented in triplets as described by Hummel et al. (1997), one containing diluted *n*-butanol and two containing the solvent (aqua conservans) only, serving as blanks. In this three-alternative forced-choice procedure, participants are asked to identify the pen containing the odorant. Each pen is presented for approximately 3 s at 1–2 cm distance of both nostrils. The interval between triplets is approximately 30 s. During testing, participants are blindfolded to avoid visual identification of the correct pen. We established ODTs based on the standard SSP procedure, an additionally computed threshold score with less reversals from the standard procedure, and the BAP.

In the standard SSP, odorants are presented from lowest to highest odor concentration. Two subsequent correct identifications trigger the first turning point (reversal of the staircase), thereby indicating the peri-threshold region. From there, odor concentration is increased following two correct answers in a row and decreased following an incorrect answer. Each turning point results in a reversal of the staircase. Seven reversals must be obtained in the "gold standard" SSP (Hummel et al. 1997). The short SSP follows the principle of the standard SSP but we estimated the threshold using only the first five of the seven measured reversals from the standard SSP.

In the BAP, each triplet is presented only once in an ascending order from lowest to highest odor concentration. The threshold score is defined as the point of transition between no detection and detection of the odorant, i.e., the threshold score is a value read at the boundary between correct and incorrect detection of the pen containing the odor. Based on the CSP (Fechner 1860) mentioned earlier, we presented each odor level only once. Similarly, based on the ALP (Cain et al. 1988), we defined the threshold score as being reached after five correct odor detections in a row. If the series of five correct detections begins within the five highest odor concentrations, the highest concentration level is repeated until five correct detections are reached unless the highest odor concentration is not detected, in which case the threshold value is zero.

Questionnaires and Interviews

Depressive symptoms were assessed using the Beck Depression Inventory (BDI) (Beck et al. 1961), a self-administered four-point rating scale (0 = not at all to 3 = always), which measures depressive symptoms in the past week, in order to exclude participants with depressive symptoms because depression has previously been shown to be associated with smell impairments (Croy and Hummel 2017).

Due to known effects of smoking on the olfactory system, smoking behavior was investigated using the Fagerstroem Test for Nicotine Dependence (FTQ, Fagerstroem 1978) as well as a smoking interview implemented previously in the Leipzig Life-Study containing questions about smoking behavior in the past and present, smoking onset and durations, breaks, and passive smoking hours (Loeffler et al. 2015).

To measure individual odor associations, use of the olfactory sense and the way olfaction influences decisions in daily life; we implemented the Importance of Olfaction Questionnaire (IOQ) (Croy et al. 2010).

Women were further interviewed to assess information about their menstrual cycle, because sensitivity to odors is known to be increased in follicular phase of the cycle/under oral contraceptive and decreased in luteal phase (Derntl et al. 2013; McNeil et al. 2013).

Data Analysis

JASP (version 0.8.1.1 for Mac OS X, JASP Team 2018), IBM SPSS (IBM Corp. Released 2015, IBM SPSS Statistics for Windows, Version 23.0.) and R (version 3.5.0, R Core Team 2013) were used for statistical evaluation. The α-level was set at .05.

Due to small sample size, normality of the data was ascertained using the Shapiro-Wilk test.

Age, BMI, ODT scores (SSP_7, SSP_5 on both days; BAP on the first day), perceived intensity, as well as pleasantness and familiarity of *n*-butanol on both days were normally distributed. ODT scores measured with BAP on the second day were not normally distributed. Although ANOVAs are relatively robust against violations of the assumption of normality, we nevertheless decided to perform each analysis, which included BAP threshold on day two, additionally with nonparametric testing as a precaution. As nonparametric test results did not deviate from parametric test results, we decided to report the latter here.

Data obtained with the "gold standard" SSP were analyzed twice. In a first step, we computed the standard ODT score, which is calculated by the mean of the last four of a total of seven reversals (SSP_7). A second threshold score was computed by the last two of a total of five reversals (SSP_5).

For BAP, the threshold value was estimated by identifying the point of transition between no detection and detection, which means, the point when an odorant was constantly detected five times in a row.

To compare the ODT scores obtained with the "gold standard" SSP with seven reversals (SSP_7), the short SSP with five reversals (SSP_5), the BAP, and between testing days, the data were submitted to repeated-measures analysis of variance (rm-ANOVA) using the general linear model with the within-subject factors "Method" (SSP_7/SSP_5/BAP) and "Test day" (T1/T2) and the between-subject factor "Sex" (male/female). Subsequently, we ran a Bayesian repeated measures analysis of variance (Bayesian rm-ANOVA) using the same model to ascertain that there are no significant differences between the ODT scores obtained with the different methods. While conventional statistical testing is based on the frequentist paradigm, the Bayesian approach is based on the subjective probability paradigm (van de Schoot et al. 2014). Compared to conventional statistical testing, the Bayesian approach is advantageous in that the likelihood of an outcome is considered under the null *and* the alternative hypothesis. This means that by using the Bayesian approach, we can actually estimate the probability of the null hypothesis (no differences between groups in our case), while in the conventional approach, we can only estimate the likelihood of our observations or more extreme values when the null hypothesis of no differences is true.

To examine the test-retest reliability, meaning the relationship between all ODT scores obtained with different methods and on different testing days, we used intraclass correlation (ICC). ICC estimates were calculated using SPSS statistical package version 23 (SPSS Inc., Chicago, IL) based on an absolute-agreement, two-way mixed-effects model. Additionally, we report Pearson's correlation coefficients to make our results comparable to other test-retest correlation studies in olfactory testing. To describe advantages regarding relevant time-saving of the short over the standard procedure, we used rm-ANOVA based on *p* values with the within-subject factors "Method' (SSP_7/SSP_5/BAP) and "Test day" (T1/T2) and the between-subject factor "Sex" (male/female).

To compare the differences of the interindividual variation regarding the duration of the three methods in order to find out whether the stability of the implementation time differs according to the assessed method, we first computed the three different Coefficients of Variation (CV), then adjusted the CVs for the mean of each method, and finally performed a one-way ANOVA with the dependent variable "adjusted CV."

Results

Sample Characteristics

BDI scores indicated no or only mild depressive symptoms in all subjects (mean = 6.45, SD = 4.86, range 0–17). All participants were nonsmokers (assessed with Fagerstroem scale), and none declared being ex-smokers. Passive smoking hours were the following: mean = 5.58 h/week, SD = 11.60 h/week, range 0–48 h/week. The questionnaire about the individual importance of odors showed the following results: sum-score (mean = 55.35, SD = 5.25, range 45–66), association-scale (mean = 18.10, SD = 2.59, range 13–23), application-scale (mean = 16.65, SD = 3.03, range 8–21), consequences-scale (mean = 16.60, SD = 2.33, range 11–20). No significant correlations between BDI score, passive smoking hours, or olfaction scales with ODTs were observed. Furthermore, no significant differences between men and women (SSP_7 $F = .251$, $p = .284$; SSP_5 $F = .521$, $p = .480$; BAP $F = .850$, $p = .369$; ANOVA) were observed. Perceived pleasantness, intensity, and familiarity of *n*-butanol are presented in Table 1. Odorant ratings did not differ significantly between days ($F = .004$, $p = .948$; rm-ANOVA).

Test Duration

Mean test duration is presented in Fig. 1. Test duration differed significantly between methods (rm-ANOVA, main

Table 1 Visual analog ratings for *n*-butanol

Quality	VAS-rating day 1	VAS-rating day 2	*p* value
Pleasantness	3.7 ± 2.0	3.8 ± 1.8	NS
Intensity	8.0 ± 1.4	7.4 ± 1.6	NS
Familiarity	6.6 ± 2.6	6.8 ± 2.2	NS

NS not significant

effect "Method": df = 2; $F = 143.15$, $p < .001$; post hoc t tests revealed significant differences between test duration for all three methods $p < .001$). The average trial number needed for threshold determination was 21.78 (SD = 2.75) trials for the standard SSP_7, 15.98 (SD = 2.71) trials for the short SSP_5, and 12.80 (SD = 1.58) trials for the BAP. The interindividual difference of test duration did not significantly differ between methods, this means, no method is more stable in terms of duration than the other (coefficient of variance for SSP_7 = 12.6%, SSP_5 = 17.0%, BAP = 12.5%; one-way ANOVA, main effect "SD of different methods": df = 2; $F = 1.281$, $p = .286$).

Validity of BAP and SSP_5

The threshold scores of the three methods did not differ (Fig. 2). The rm-ANOVA based on p values showed no significant differences between the three methods (main effect "Method": df = 2; $F = 1.328$, $p = .278$; interaction "Method" × "Testday": df = 2; $F = 1.460$, $p = .243$). Expecting no differences between the threshold scores of the three methods, we also estimated a Bayes factor using Bayesian information criteria (Wagenmakers 2007) in order to estimate the likelihood that the null hypothesis holds. The Bayesian rm-ANOVA showed moderate evidence in favor of the null hypothesis for a main effect of "Method" ($BF_{01} = 3.788$), that means, it is 3.8 times more likely that there is no difference between the ODT scores (null hypothesis) than that there is a difference (alternative). Furthermore, there is strong evidence in favor of the null hypothesis for the interaction "Method" × "Testday" ($BF_{01} = 25.319$), that is, it is 25.3 times more likely that for each method, there is no difference of ODT scores between days.

Further, correlation coefficients between all short procedures and the standard SSP were significant (Table 2) with a high positive relationship between SSP_7 and SSP_5 as well as a moderate positive relationship between SSP_7 and BAP showing the interrelation between the different methods. Moreover, the two short procedures were highly correlated ($r = .696$, $p = .001$).

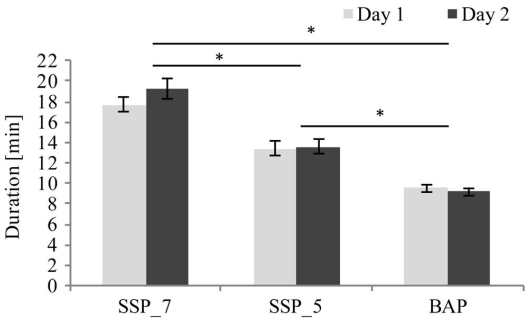

Fig. 1 Mean test duration of the three different methods

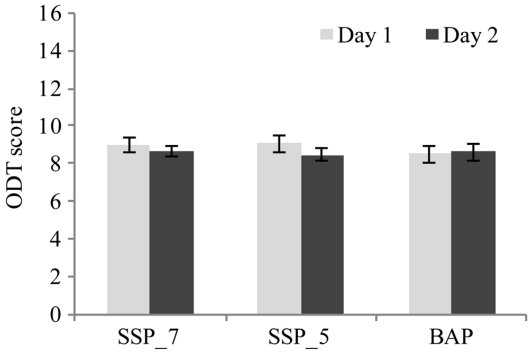

Fig. 2 Mean threshold scores of the three different methods

Test-Retest Reliability

Test-retest correlation analysis of the thresholds on different test-days showed significant correlation coefficients (Table 3, Fig. 3) with intraclass correlation as well as with Pearson's correlation. We show a moderate reliability for SSP_7 and BAP respectively and a poor reliability for SSP_5, meaning a moderate positive relationship between SSP_7 as well as for BAP between test tests and a weak positive relationship for SSP_5 between test days.

Discussion

In order to establish a brief test for measuring olfactory sensitivity, we compared two short procedures with the standard ODT test, all carried out using the "Sniffin' Sticks" ODT test kit. Our aim was to provide an ODT test that is easy to administer, requires little cognitive resources of the research participant or patient population, and shows predictable and stable test duration to be used in complex study designs and in the clinical context. We showed that both alternative ODT tests are significantly shorter than the commonly used SSP. The BAP takes only half of the time the standard SSP takes, and shows a smaller, however not significantly different variability in test duration.

Moreover, measured threshold scores do not differ between all three methods, that means, the short versions result in scores comparable to those obtained with the standard SSP.

Table 2 Correlation analysis of the short testing procedures with the standard single staircase procedure

	SSP_7	SSP_5	BAP
Correlation* with standard SSP_7 day 1	1	0.98	0.76
p value	–	< .001	< .001

*Pearson's correlation coefficient

Table 3 Test-retest reliability: intraclass correlation and Pearson's correlation coefficients

	SSP_7	SSP_5	BAP
Intraclass correlation coefficient	.64	.40	.63
p value	.001	.029	.001
Pearson's correlation coefficient	.68	0.45	0.68
p value	< .001	.050	.002

The test-retest reliability measured with intraclass correlation is similar in the standard ($r = .64$, $p = .001$) and BAP procedure ($r = .63$, $p = .001$), and smaller in the short SSP_5 ($r = .40$, $p = .029$). This means that the standard and the BAP procedure show a moderate reliability, meaning that the procedures produce scores that are relatively stable over time. The SSP_5 shows a poor test-retest reliability. However, compared to other test-retest reliability analysis in olfactory testing (measured with Pearson's correlation coefficients), all three test-retest correlation coefficients are equivalent to those normally found for odor sensitivity tests, which range from 0.43–0.85 ($p < .0001$) (Albrecht et al. 2008; Hummel et al. 1997; Lotsch et al. 2004). Moreover, the mean threshold scores did not differ between testing orders, test days and age. We did not find any differences for gender, which might be due to small group sizes. Furthermore, we did not find a correlation between olfactory performance and passive smoking hours as well as subjective importance of odors assessed via questionnaires.

To sum up, the two short procedures yield ODT scores like those obtained through the standard procedure, with similar test-retest reliability in all three procedures. Moreover, the BAP is 51% and the SSP_5 26% faster than the standard procedure.

Limitations

As mentioned earlier, the test-retest reliability in all three test procedures is comparable to that typically found for ODTs. However, the coefficients indicate a moderate (SSP_7, BAP), or even poor (SSP_5) reliability (Koo and Li 2016). The reliability in olfactory testing is generally rather low, possibly because of the susceptibility of our olfactory sense to many external factors. These include smoking (Hayes and Jinks 2012), modality of odor presentation (Sorokowska et al. 2015), hunger state (Albrecht et al. 2009; Ramaekers et al. 2016), menstrual cycle (Derntl et al. 2013; McNeil et al. 2013), climate (Katotomichelakis et al. 2007), and altered state of the nasal epithelium trough virus susceptibility according to the season (Konstantinidis et al. 2006). We here attempted to counteract those influences by controlling for several confounding factors (smoking, season, cycle phase, hunger). Nonetheless, we were unable to address all possible confounders satisfactorily, in particular hunger state. While we advised participants to refrain from eating 2 h prior to testing to avoid being either hungry or sated, we did not control food intake or quantify hunger state. For future studies, we would recommend using visual analog scales to assess feelings of hunger and satiety and, if possible, providing a standardized meal at the test institute.

Additionally, the resolution of the new BAP method is lower than the SSP, as it produces whole numbers only, whereas the thresholds computed of the SSPs give decimals. The BAP is therefore convenient in the clinical context when expecting large group differences, but might not be suitable in complex research designs to find small group differences between healthy populations for example according to different background odor stimulation or hormonal changes during pregnancy. Similarly, the BAP is more error prone than the SPPs—the impact of false positives/false negatives on the actual threshold score is higher in the BAP because each odor concentration step is presented only once. Moreover, since we derived two threshold values (SSP_7 and SSP_5) from the single staircase procedure, the true correlation is over-estimated because they are highly dependent.

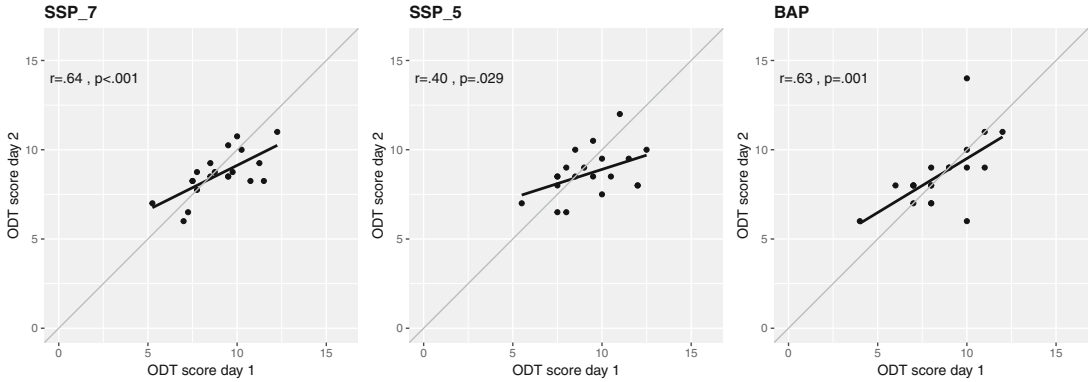

Fig. 3 Test-retest correlations for all three methods. The gray line represents the identity line ($y = x$)

Nonetheless, these limitations do not detract from the main advantages of the BAP: its brevity and more stable test duration, which can be of crucial importance in complex study designs with limited testing time. This is also an advantage for patients with limited cognitive resources, such as attention deficits. Another great advantage of the BAP compared to the SSPs is that it is easier to use. The odor concentrations are presented in an ascending order without any turning points and jumps, making the method less prone to errors from the investigator's side.

Conclusion

In this study, we assessed validity and reliability of standard and short ODT procedures to test olfactory sensitivity. We show that the short BAP is a valid and stable method and a good alternative to the standard SSP. While it is less precise and more susceptible to the influence of type one and two errors, it is also much shorter than the standard SSP. Although the task requires the same amount of effort within one trial under all three conditions regarding memory, having this demanding and exhausting task shortened 51% or 26% is very helpful for staying attentive and motivated to complete the task successfully. Moreover, especially the BAP method is also very easy to assess for the investigator and can thereby be used in the stressful daily clinical routine without further aids (computer software; paper template sheets). All three methods are easy to assess with the prefabricated, commercially available "Sniffin' Sticks."

Hence, we recommend using the BAP if only a limited time frame for testing is available or if examining patients/participants with limited cognitive resources.

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Compliance with Ethical Standards

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Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval and Informed Consent The study was carried out in accordance with the Declaration of Helsinki and was approved by the Local Ethics Committee. The research involved human participants. They provided written informed consent.

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Study 2: Odor sensitivity for food and non-food odors in obesity

Insulin resistance is associated with reduced food odor sensitivity across a wide range of body weights.

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Insulin Resistance Is Associated with Reduced Food Odor Sensitivity across a Wide Range of Body Weights

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Abstract: The worldwide obesity epidemic is a major health problem driven by the modern food environment. Recently, it has been shown that smell perception plays a key role in eating behavior and is altered in obesity. However, the underlying mechanisms of this phenomenon are not well understood yet. Since the olfactory system is closely linked to the endocrine system, we hypothesized that hormonal shifts in obesity might explain this relationship. In a within-subject, repeated-measures design, we investigated sensitivity to a food and a non-food odor in the hungry and sated state in 75 young healthy (26 normal weight, 25 overweight, and 24 obese) participants (37 women). To determine metabolic health status and hormonal reactivity in response to food intake, we assessed pre- and postprandial levels of insulin, leptin, glucose, and ghrelin. Odor sensitivity did not directly depend on body weight status/body mass index (BMI) or hunger state. However, we could establish a strong negative mediating effect of insulin resistance on the relationship between BMI/waist-hip ratio and olfactory sensitivity for the food odor. These findings indicate an impact of metabolic health status on sensitivity to food odors. Our results contribute to a better understanding of the mechanisms behind altered smell perception in obesity.

Keywords: obesity; odor sensitivity; olfaction; HOMA-IR; insulin resistance

1. Introduction

The obesity epidemic is a major health problem that is associated with severe comorbidities such as diabetes, stroke, and cancer [1,2]. Although causal mechanisms and possible treatment approaches

are being studied intensively, the occurrence of overweight as defined by a body mass index (BMI) between 25 and 29.9 kg/m^2 and obesity (BMI > 30 kg/m^2) has almost tripled within the last 40 years [3]. Currently, obesity has a worldwide prevalence of 13% and overweight of 39% [3].

Olfaction in obesity: While the etiology of obesity is multifactorial, one of the main contributing factors driving this rapid increase is the obesogenic environment [4,5]. Our environment is full of energy-rich foods that are advertised via stimuli for all sensory channels. Importantly, the sense of smell plays a crucial role in eating behavior and influences food choice and meal size [6,7]. Individuals with obesity more than those of normal weight are susceptible to external food cues such as food pictures [8–11] but also food smells [12]. Moreover, individuals with obesity perceive food odors as more pleasant than people of normal weight [13], while being surprisingly less sensitive to odors [14,15]. Previous studies showed that olfactory performance with respect to identification and discrimination of odors, as well as perceptual sensitivity, is low in obesity [15]. The majority of these studies used the Sniffin' Sticks with a standard olfactory detection threshold (ODT) test, which contains the non-food odors n-butanol, a rather unpleasant odor that naturally forms during fermentation, or phenylethyl alcohol (rose-like odor). Given the body of literature suggesting a negative relationship between BMI and olfactory capacity, researchers postulated diverse mechanisms of metabolic and neural malfunction in obesity [16,17]. However, Stafford and Whittle [13] showed recently, that individuals with obesity compared to those of normal weight show a higher sensitivity to the smell of chocolate. Accordingly, smell capacity might not be quantitatively impaired, but qualitatively altered. Since there is no standardized test for assessing chocolate smell sensitivity, we developed a chocolate odor test kit which is similar to the standard Sniffin' Sticks in terms of odor concentrations and dilution steps. We decided to use chocolate as a food odor as the smell of chocolate has previously been associated with food cravings [10].

Physiological status and odor sensitivity: In our environment, odors signal the availability of food. Therefore, odor sensitivity may also depend on the hunger status (hungry vs. sated), a notion that has been clearly shown in animals before: rats show enhanced sniffing behavior and higher sensitivity to odors [18] in the fasted when compared to the sated state. In humans, however, results are divergent and show both higher [19] and lower sensitivity [20] in fasted states, or no difference at all [21]. While these studies predominantly used non-food odors, we reasoned that food odors are more relevant in the context of obesity. Thus, we investigated differential effects of food as compared to non-food odors in the hungry and sated state.

Hormones and the olfactory system: Alternatively, metabolic and hormonal differences between study populations might explain controversial results in odor sensitivity. For instance, study populations with obesity class 1 (BMI 30–34.9 kg/m^2) and class 3 (BMI ≥ 40 kg/m^2) are afflicted differently by hyperinsulinemia, hyperleptinemia, insulin and leptin resistance or low ghrelin levels [22,23]. Interestingly, Palouzier-Paulignan [24] introduced a complex hormonal and metabolic model that provides a neuroanatomical and -physiological link between the olfactory and endocrine systems. Especially the olfactory mucosa and the olfactory bulbs show a high density of insulin, leptin, and ghrelin receptors [25,26], hormones that are actively involved in signaling and regulating the homeostatic state and modulate odor sensitivity [27]. From this it can be concluded that these hormones might be strong modulators of olfactory perception. Thus, we investigate pre- and postprandial levels of insulin, glucose, leptin, and ghrelin and relate these measures to odor sensitivity.

Obesity and hormones: Individuals with obesity show several hormonal changes that are possibly leading to an altered diet and internal processing of foods. The influence of these hormones on eating behavior and related conditions such as obesity have been intensively studied in recent years [28]. While orexigenic hormones such as ghrelin and adiponectin stimulate appetite and food intake, anorexic hormones such as insulin and leptin induce satiety and regulate long-term energy homeostasis [29]. Obesity is associated with higher levels of insulin and leptin [30,31], while sensitivity to these hormones is reduced [32,33]. In addition, the plasma total ghrelin levels and ghrelin reactivity are lower in individuals with obesity when compared to those of normal weight [22,23,34]. Typically, ghrelin

decreases after eating in healthy normal weighted individuals [35]. Since the ghrelin response to food intake is blunted in obesity, ghrelin might act independent of attenuated physiological needs in obesity, because ghrelin level does not properly decrease after a meal. Accordingly, individuals with obesity might experience unaffected high appetite after eating. For ghrelin, an acylated (active) and unacylated (inhibiting) form exists [36]. Of particular interest is the elevated ratio of acylated (AG) to unacylated ghrelin (UAG) in obesity [37]. It is pivotal for maintaining weight balance [36], since an elevated AG/UAG ratio could reflect the lower level of UAG and thus be responsible for a consistently high appetite and urge to eat even after a meal.

Summary: Within this study we aim to explore whether the effects of weight status and hunger state on olfactory sensitivity are mediated by endocrine changes in obesity. Based on current evidence, we assumed that participants of normal weight would outperform those with obesity for the non-food odor and vice versa for the food odor. Second, we hypothesized that while individuals of normal weight have a lower odor sensitivity to food odors when they are sated, individuals with obesity would not show this change. Third, we assumed that the endocrine profile of participants with obesity is characterized by high insulin resistance, high leptin levels, elevated AG/UAG ratio, and low total ghrelin levels. We further expected that the endocrine profile would mediate the relationship between BMI and olfactory sensitivity.

2. Materials and Methods

2.1. Subjects

The sample consisted of 84 participants. We excluded n = 9 due to stuffed noses, poor veins, and insufficient intake of calories/satiation in the sated condition. Thus, data from 75 participants were analyzed. All participants were recruited from the Max Planck Institute database. They were aged between 18 and 35 years (27.2 ± 3.7 years) to exclude age effects on olfactory performance [38] and their BMI ranged from 18.8 to 44.2 kg/m^2. Exclusion criteria included smoking, recent history of smoking (<3 years abstinence), vegetarian/vegan diet, allergies, alcoholism (reported intake > 5 times per week), pregnancy/breastfeeding, nose surgery except childhood polypectomy, and history of neurological/psychiatric disorders. Participants provided written informed consent. The study was carried out in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the University of Leipzig (170-16/ek-25042016).

2.2. Study Design

On two consecutive days, the study participants came to their testing at the same time each day. They fasted overnight (approx. 12 h) and received a meal on the first or second day in pseudo-randomized order (Figure 1). The meal consisted of a cereal-fruit-smoothie (either banana or wild berry) with 25% of the participants' daily energy requirement determined by an interview about their physical activity level and body weight/height. All participants were screened for olfactory function via the short form [39] of olfactory identification test. They underwent a medical examination to assess body weight, height, waist, and hip circumference as well as several interviews. On both test days, participants rated intensity, pleasantness, and familiarity of the odorants that were applied for odor sensitivity testing on visual analogue scales [40] (How strong or intense is this odor?/0 = very weak, 10 = very strong; How pleasant/unpleasant is this odor?/−5 = unpleasant, +5 = pleasant; How familiar is this odor?/0 = unfamiliar, 10 = familiar). We collected blood samples on both test days in the morning and 60 min after meal onset/break without meal. Thirty minutes after meal onset/break without meal, participants performed ODT tests for the three odors (food pleasant: chocolate; non-food pleasant: grass; and non-food standard: n-butanol) in pseudo-randomized order.

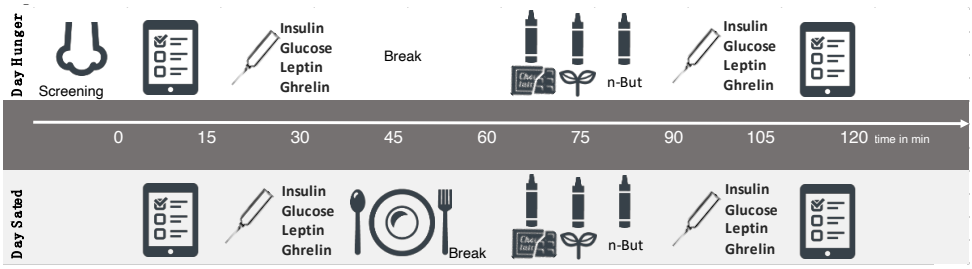

Figure 1. Study Design.

2.3. Questionnaires and Interviews

Depressive symptoms were assessed using the Beck Depression Inventory (BDI) [41] in a paper pencil form before olfactory testing to directly control for acute suicidal tendencies and exclude participants with more than mild depressive symptoms (>score 18). Additionally, before starting the study protocol, the participants were face to face interviewed about their smoke status as an additional control to the telephone interview to exclude people according to our exclusion criteria on smoking. The conducted smoking interview was previously used in the Leipzig Life Study, and included questions on smoking behavior in the past and present, on the beginning and duration of smoking, on breaks and passive smoking [42]. Women were further interviewed to obtain information about their menstrual cycle, because sensitivity to odors is increased in follicular phase of the cycle/under oral contraceptive and decreased in luteal phase [43,44].

2.4. Testing of Olfactory Performance

All odorants were presented in commercially available felt-tip pens (Sniffin' Sticks; Burghart Instruments, Wedel, Germany). For the testing of olfactory sensitivity, the ODT test-kit from the Sniffin' Sticks test battery was implemented for the standard non-food odor. The kit consists of a geometric series of sixteen dilutions of n-butanol. We further developed a test-kit for a sweet, high fat food odor (chocolate). As a pilot study indicated that the standard test odor n-butanol is perceived as rather unpleasant, we decided to develop a second test-kit with a pleasant non-food odor (freshly mown grass). We therefore filled the odorless tampons of blank Sniffin' Sticks with either chocolate odor (chocolat noir, code:1130/4, Givaudan) or grass (cis-3-hexen-1-ol, code: H12900-1OG, Aldrich). Based on the common n-butanol test we developed a similar geometric series of sixteen dilutions for each odor. To obtain similar perceived intensity of the three odors, chocolate pens were filled with highest 1% and lowest 31 ppm and grass pens were filled with a highest odor concentration of 4% and the lowest of 1.22 ppm, both in a similar ratio of 1:2 (pilot study, $n = 10$). We then applied the short single staircase procedure as described in Poessel et al. [45] to determine odor thresholds.

2.5. Blood Collection

Blood samples were collected after 12 h of overnight fasting in vacutainer tubes treated with ethylenediaminetetraacetic acid (EDTA) for serum (insulin, leptin) or with aprotinin (AG, UAG, and total ghrelin). Serum was collected in 7 mL Sarstedt monovettes. After 30 min, tubes were centrifuged at 4000 r.p.m. at 4 °C. The serum was separated in two 1 mL tubes (Eppendorf Safe-Lock Tubes) and immediately frozen at −80 °C. Blood for glucose determination was collected in 2.7 mL glucose monovettes and treated as serum tubes. Acylated and unacylated ghrelin was measured by ELISA easy sampling kit (Bertin Pharma, Montigny-le-Brettoneux, France) and total ghrelin was measured by ELISA kit (Millipore Corporation, MA, USA). Blood for ghrelin was collected into 5 mL aprotinin tubes to avoid hormone degradation, which was then put on ice immediately at 2–4 °C for max 15 min. Tubes were centrifuged for 10 min at 3500 rpm at 4 °C and after pipetting in two 0.5 tubes immediately stored at −80 °C.

2.6. Data Analysis

R version 3.4.3. within RStudio [46] and SPSS (version 22.0, SPSS Inc., Chicago, IL) were used for statistical evaluation. We used BMI as a grouping variable (normal weight: BMI 18.4–24.99 kg/m^2, overweight: BMI 25.0–29.99 kg/m^2, and obese: BMI > 30 kg/m^2) or as a continuous variable. We used waist–hip ratio (WHR) as an additional measure of weight status that is more related to metabolic health and reflects visceral fat status. As an estimate of insulin resistance, we used the homeostasis model assessment-insulin resistance (HOMA-IR, [47]), applying the formula: HOMA-IR = glucose (mmol/L) × insulin (pmol/L)/135. The α-level was set at 0.05. We used Bonferroni correction to adjust the α-level for multiple testing. Whenever statistical assumptions for parametric testing were violated, we applied non-parametric robust tests. We defined outliers as values below or above 2.2 interquartile range from the samples lower or upper quartile [48]. We used standardized values (z-transformation) whenever we directly compared the three odorants, because they slightly differed in perceived intensity. To compare food vs. non-food odor condition, we used chocolate as food and only grass as non-food odor as they were perceived as similarly pleasant. To depict change over time we applied a residualized change model instead of the difference score model to avoid the regression to the mean phenomenon [49]. We used multivariate ANCOVA to depict differences between BMI groups (=between subject variable) for the three ODT values as within subject variables, using "sex" as a covariate. Assumption tests showed homogeneity of variances/covariances, as assessed by Box's M test. There were no univariate/multivariate outliers, as assessed by standardized residuals greater than ± 3 standard deviations/Mahalanobis distance. Residuals were normally distributed for chocolate and n-butanol, but not for grass odor sensitivity. However, we decided to apply the one-way MANCOVA since it is robust to deviations from normality. Further, we applied a two-way mixed repeated measures ANCOVA to depict the influence of hunger state on olfactory performance. Having a 3 × 2 × 2 design, weight groups (normal weight, overweight, and obesity) were used as between-subject factor; ODT scores (food vs. non-food) and hunger status (hungry vs. full) as within-subject factors and "sex" as a covariate. There were three outliers. Thus, we performed our analysis with and without the outliers. Since results of both analyses did not differ, we decided to keep the outliers within our analysis. ODTs were normally distributed in the hungry, but not in the sated state. Since ANOVAs are robust to deviations from normality, we decided to run this analysis anyway. We used mediation analysis to explore the processes that might underlie the relationship between BMI and odor sensitivity by introducing a third variable: the hormonal status. Unstandardized indirect effects were computed for each of 10,000 bootstrapped samples, and the 95% confidence interval was computed by determining the indirect effects at the 2.5th and 97.5th percentiles. Additionally, we repeated the mediation using WHR as independent variable to investigate the influence of visceral fat on olfaction. For a post-hoc test we grouped our data according to insulin resistance (NH: normal HOMA-IR ≤ 1; EH: elevated HOMA-IR > 1). Since there is no absolute value for HOMA indices and no fixed classification of "normal" and "abnormal" values, our grouping relies on recent data from Lee et al. [50].

3. Results

Participant characteristics, hormonal parameters that are associated with metabolic health, ODT scores, and odorant ratings are depicted in Table 1. BDI scores indicated no depressive symptoms in all participants. All participants were non-smokers. The weight groups differed in their endocrine profile. Participants with overweight and obesity showed significantly higher HOMA-IR scores and leptin levels than participants with normal weight. Total ghrelin was lower in participants with obesity when compared to participants with normal weight/overweight. AG/UAG ratio did not differ between groups (for more detail see Table S2). Odorant ratings did not differ between groups.

Table 1. Participant characteristics, metabolic and endocrine profiles, odor detection thresholds, and odorant ratings.

	Total	Normal Weight (NW)	Overweight (OW)	Obese (OB)	*p*-Value
	(75, 37 females)	(*n* = 26, 14 females)	(*n* = 25, 12 females)	(*n* = 24, 11 females)	
Characteristics					
Age (years)	27.2 ± 3.7	26.1 ± 2.7	27.3 ± 1.3	27.7 ± 4.4	$p = 0.142$ [a]
BMI (kg/m^2)	27.8 ± 5.3	22.4 ± 1.7	27.3 ± 1.3	34.1 ± 3.5	$p < 0.001$ [a]
BDI sum	4.0 ± 3.6	2.9 ± 3.0	4.5 ± 4.1	4.5 ± 3.4	$p = 0.169$ [a]
Passive Smoke	3.0 ± 8.5	4.8 ± 13.9	2.1 ± 2.7	2.1 ± 3.5	$p = 0.443$ [a]
Metabolic profile					
HOMA-IR	1.18 ± 0.79	0.71 ± 0.30	1.11 ±0.63	1.78 ± 0.94	$p = 0.019$ [b], $p < 0.001$ [c], $p = 0.018$ [d]
Leptin	15.59 ± 17.88	6.19 ± 3.92	12.65 ± 13.1	26.77 ± 23.25	$p < 0.001$ [a]
Total ghrelin	571.53 ± 218.17	604.35 ± 238.32	626.46 ± 186.43	478.75 ± 204.12	$p = 0.926$ [b], $p = 0.097$ [c], $p = 0.044$ [d]
AG/UAG ratio	19.92 ±14.21	21.39 ± 16.25	16.22 ± 8.67	22.19 ± 16.17	$p = 0.278$ [a]
Odors					
N-butanol					
Pleasantness		3.72 ± 1.80	3.23 ± 1.71	4.14 ± 2.03	ns [a]
Intensity		7.28 ± 1.78	7.52 ± 1.80	6.67 ± 1.88	ns [a]
Familiarity		5.72 ± 2.51	6.00 ±2.79	5.16 ± 2.65	ns [a]
Chocolate					
Pleasantness		8.03 ± 1.46	7.76 ± 2.04	7.55 ± 1.54	ns [a]
Intensity		7.99 ± 1.70	7.79 ± 1.64	7.79 ± 1.45	ns [a]
Familiarity		8.61 ± 1.32	8.80 ± 1.24	8.26 ± 1.65	ns [a]
Grass					
Pleasantness		7.31 ± 1.55	7.46 ± 1.92	6.58 ±2.51	ns [a]
Intensity		7.73 ± 1.63	7.80 ± 2.05	7.64 ± 1.70	ns [a]
Familiarity		7.69 ± 2.10	7.90 ± 2.83	7.35 ± 2.71	ns [a]

Abbreviations: BMI—body mass index; BDI—Becks Depression Inventory; Passive Smoke—passive smoking hours per week; HOMA-IR—homeostatic model assessment—Insulin Resistance; AG/UAG ratio—ratio of acylated to unacylated ghrelin [a]—between all groups; [b]—between NW and OW group; [c]—between NW and OB group; [d]—between OW and OB group.

3.1. Main Hypotheses

3.1.1. Hypothesis 1: General Olfactory Sensitivity Differs Between Weight Groups Depending on Odor Quality

We determined the effect of body weight status on olfactory sensitivity for chocolate, grass, and n-butanol with a one-way MANCOVA. We could show that there was no statistically significant difference between the weight groups on odor thresholds, $F(2,138) = 0.004$, $p = 0.881$, Wilks' lambda = 0.967, and partial eta^2 = 0.017 (Figure 2).

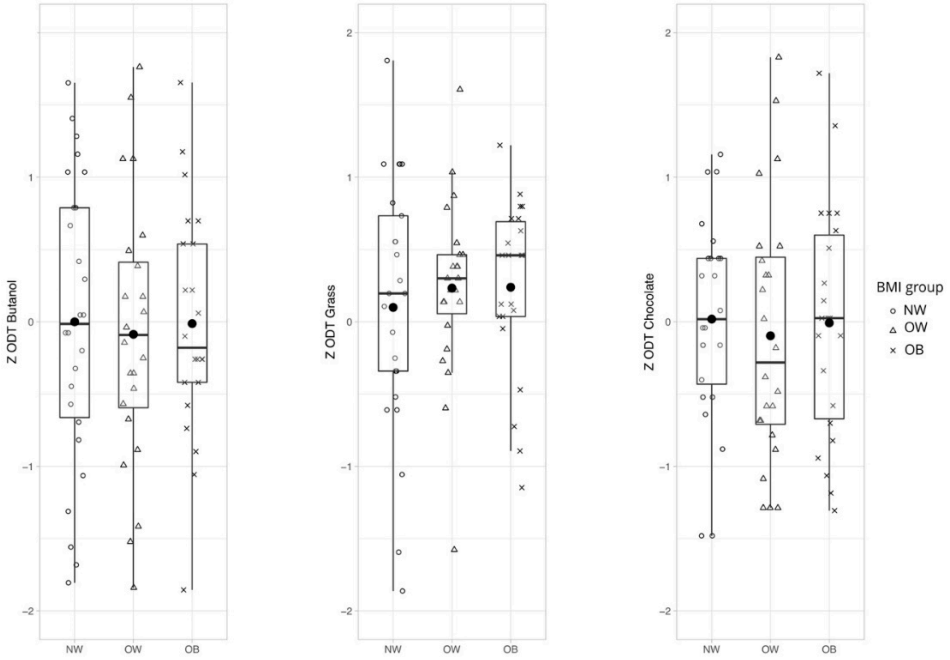

Figure 2. Hypothesis 1: General odor sensitivity for chocolate, grass, and n-Butanol in different weight groups. No statistically significant difference between normal weight, overweight, and obese participants on odor thresholds after controlling for sex, F(2,138) = 0.004, p = 0.881. Abbreviations: NW—normal weight; OW—overweight; OB—obese; and ZODT—z-standardized score for odor thresholds.

3.1.2. Hypothesis 2: Olfactory Sensitivity Depends on Hunger State

A two-way mixed repeated-measures ANCOVA was run to determine the effect of hunger state on odor sensitivity for the food and non-food odor depending on weight status. There was no main effect of hunger state (F(1,64) = 0.189, p = 0.665, and rm-ANCOVA), meaning that being hungry or sated had no influence on odor thresholds across groups (see Figures S1 and S2 for sanity checks on the applied hunger modulation). Additionally, there was no between subjects effect for weight status (F(2,64) = 0.559, p = 0.575, and rm-ANCOVA), meaning that being hungry or sated did not differently affect olfactory performance in the three weight groups (Figure 3). Finally, there was no significant interaction between odor, hunger state, and BMI group on ODT score (F(2,66) = 1.913, p = 0.156, and partial eta^2 = 0.055).

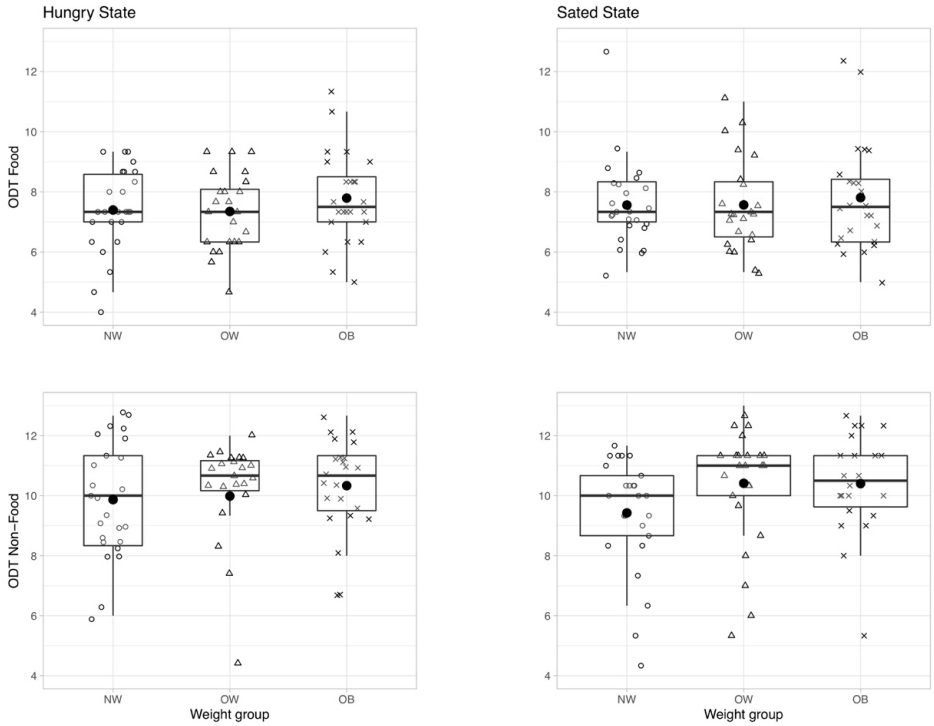

Figure 3. Olfactory detection threshold (ODT) for food (top) and non-food odor condition (bottom) according to hunger state: hungry (left) vs. sated (right); depicted for different weight groups (NW—normal weight, OW—overweight, and OB—obese). ODT ranges from score 0—highest odor concentration (= low odor sensitivity) to 16—lowest odor concentration (=high odor sensitivity). No significant main or interaction effect.

3.1.3. Hypothesis 3: Hormones Mediate Olfactory Performance

General Odor Sensitivity

The relationship between BMI and odor sensitivity for chocolate was mediated by metabolic health parameters. As Figure 4A,C illustrate, the standardized regression coefficients between BMI/WHR and metabolic health indicators were significant for IR (insulin resistance) as assessed by HOMA-IR score and leptin levels (a-path), as were the standardized regression coefficients between IR and odor sensitivity (b-path). The standardized indirect effect between BMI and odor sensitivity via IR was −0.256 (CI −0.689 and −0.026) and for WHR −0.241 (CI −0.689 and −0.026). There was no direct or indirect relationship between BMI and odor sensitivity for the non-food odor condition (Table S3).

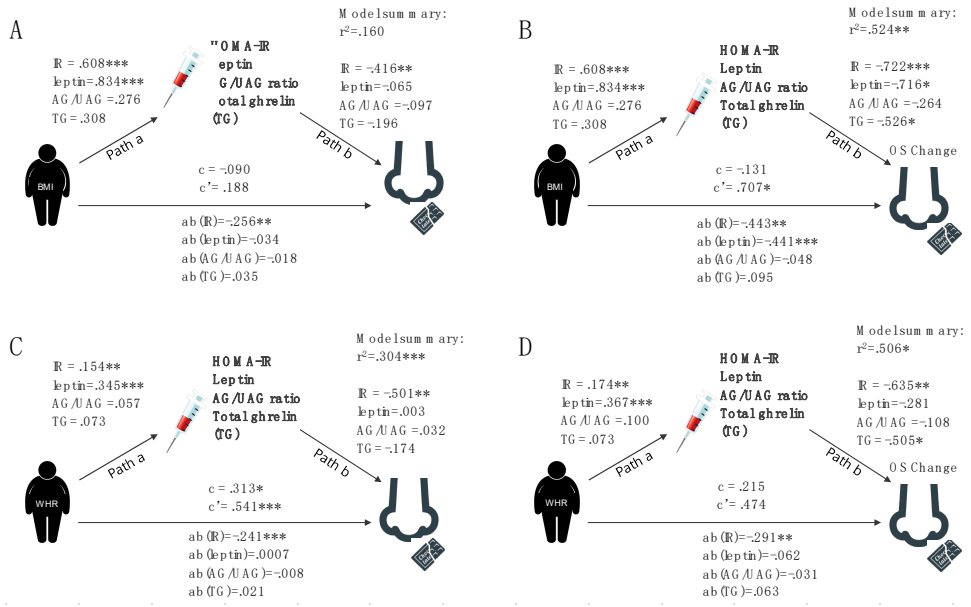

Figure 4. Mediation analysis for odor sensitivity to chocolate. (**A**) Mediation analysis for general chocolate odor sensitivity. Path a—represents the relationship between BMI and hormonal parameters; b—represents the relationship between odor sensitivity and hormonal parameters; c—represents the total effect (direct + indirect effect); c'—represents the direct relationship between BMI and odor sensitivity; ab—represents the indirect effect of hormonal parameters on the relationship between BMI and odor sensitivity. (**B**) Mediation analysis for the change in chocolate odor sensitivity (OS change) in response to meal intake. (**C**) Mediation analysis for chocolate odor sensitivity using waits–hip ratio (WHR) instead of BMI as independent variable. (**D**) Mediation analysis for the change in chocolate odor sensitivity (OS change) in response to meal intake using WHR as independent variable.

Change in Odor Sensitivity in Response to a Meal

The relationship between BMI and the change in odor sensitivity after meal intake is indirectly predicted by hormonal parameters that are related to metabolic health. As Figure 4A,D illustrate, the standardized regression coefficients between BMI/WHR and hormones were significant for IR and leptin. The b-path was significant for IR, leptin, and total ghrelin in the BMI model and for IR only in the WHR model. The standardized indirect effect was significant for IR –0.443 (CI –0.546 and –0.214) and leptin −0.441 (CI −0.512 and −0.112).

3.2. Post Hoc Analyses

To understand the role of insulin resistance in the interplay of olfaction and obesity, we performed a post hoc analysis of the hypothesis 2 model using IR as an additional covariate. Interestingly, we could now show a main effect of BMI group for the food odor $F(2, 65) = 3.303$, $p = 0.043$, and partial $\eta^2 = 0.093$ (Figure 5). Post hoc comparisons showed that individuals with obesity outperformed those of normal weight when IR was controlled (mean difference = −1.293, SE = 0.391, $t = -3.309$, and $p = 0.005$; Tukey test).

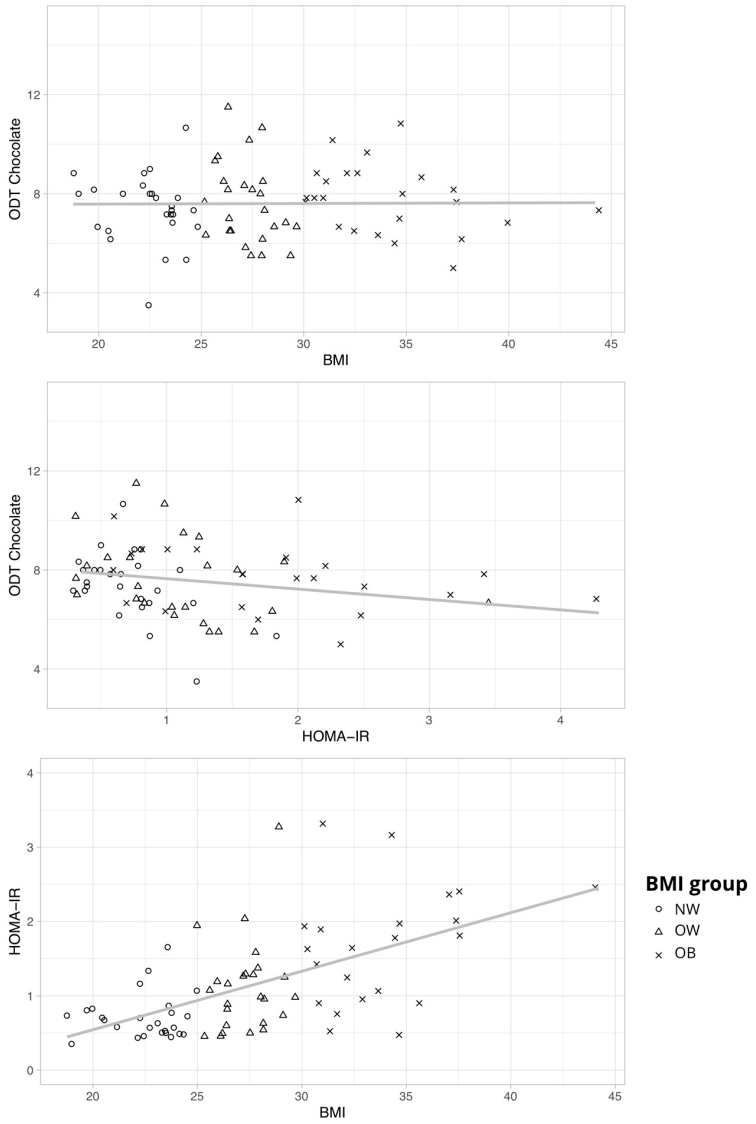

Figure 5. Scatterplots with regression lines to depict the relationship between chocolate odor sensitivity (ODT Chocolate), BMI, and HOMA-IR.

Finally, for another post-hoc analysis, participants were classified into two groups according to their insulin resistance: optimal HOMA-IR ≤ 1 (n = 41) and elevated HOMA-IR > 1 (n = 33). We performed a multivariate ANOVA using IR-group as group variable and olfactory sensitivity as dependent variable. Olfactory sensitivity was higher in the optimal HOMA-IR group (food: M = 7.9, SD = 1.3; non-food: M = 10.4, SD = 1.7) when compared to elevated HOMA-IR group (M = 7.1, SD = 1.5; non-food: M = 9.7, SD = 2.2). The difference was significant for the food odor condition, ODT food: $F(1,74) = 8.608$, $p = 0.005$, and partial $\eta^2 = 0.108$; ODT non-food: $F(1,74) = 2.613$, $p = 0.110$, and partial $\eta^2 = 0.035$. To sum up, BMI seems to be positively associated with olfactory sensitivity when controlling for IR. IR is negatively associated with olfactory sensitivity in the food odor condition independent of BMI (see Figure 6).

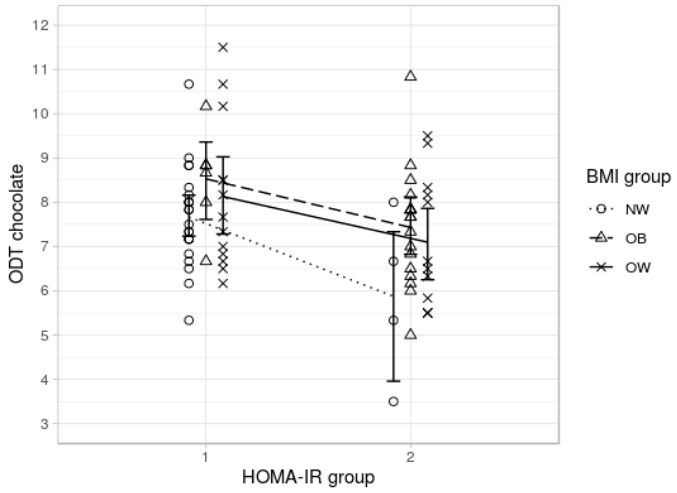

Figure 6. Chocolate odor sensitivity (ODT chocolate) for optimal (HOMA-IR < 1) and elevated HOMA-IR group (HOMA-IR > 1) with separate lines for BMI groups (NW—normal weight, OW—overweight, and OB—obese).

4. Discussion

The aim of the study was to shed light on the complex relationship between obesity and smell perception. Hence, we examined different modalities that could influence smell perception in participants with normal weight, overweight and obesity. We directly compared sensitivity to food and non-food odors and included the influence of being hungry or sated on smell sensitivity. Additionally, we assessed endocrine parameters to evaluate the metabolic health status of our participants. Since it has been shown that olfaction is directly influenced by hormones that play an important role in obesity, we related metabolic health status to our measurements of olfactory sensitivity.

In accordance with recent literature on the relationship between the olfactory and endocrine systems [24,51,52], our study shows for the first time that there is a negative indirect effect of BMI on odor sensitivity for chocolate through the metabolic health parameter insulin resistance (IR). This means that the higher the BMI the lower odor sensitivity via IR (Figure 3). With the applied mediation model, we could, as expected, show positive effects of BMI on IR and circulating leptin levels, i.e., the higher the BMI the higher IR and leptin. Furthermore, we could show that there is a negative effect of IR on odor sensitivity while controlling for BMI, i.e., the higher IR the lower odor sensitivity independent of BMI. Moreover, we could show for the first time that WHR as a measure of visceral fat mass has a direct effect on food odor sensitivity and that this effect is again mediated by IR. These findings promote suggested metabolic and hormonal mechanisms of altered smell perception in obesity [15,16]. In fact, our results offer a possible explanation for controversial findings in smell sensitivity, since most studies did not include hormonal parameters as control-variables and most likely included participants of different metabolic health status. Interestingly, the one study providing evidence of a higher smell sensitivity in obesity is the only one that examined very young and class 1 obese participants [13]. Other studies examining this relationship also included older and class 1–3 obese participants, e.g., [51,53]. (Table S4 for comparison). In the current study, the obese sample consists of 17 class 1 obese participants (BMI 30–35 kg/m^2), five class 2 participants (BMI 35–40 kg/m^2), and two class 3 participants (BMI > 40 kg/m^2). In this respect, we think it is not surprising that olfactory ability is not yet generally decreased in our sample with obesity.

In addition to this general effect, we have also established a strong mediating effect of metabolic health markers on the relationship between BMI and the change in odor sensitivity in response to meal intake. We expected that odor sensitivity decreases in response to food intake reflecting less need

to search for nutrients in the environments as has been reliably shown in animal models [18,54,55]. We assumed that this mechanism may be changed by poor metabolic health, and thus insufficient modulation of central odor sensitivity in obesity. Namely, we expected that while participants with normal weight would show a significant decrease in response to meal intake, participants with obesity might show similar odor sensitivity in the hungry and sated state in accordance with most recent literature on low reactivity of sensory (odor intensity) and metabolic (ghrelin reactivity) systems in response to changing hunger states in obesity [52]. In contrast with our expectations, we detected a negative indirect effect of BMI on change in odor sensitivity through the mediator IR, meaning that the higher the BMI together with high IR, the more negative is the change in odor sensitivity. A negative change score represents a decrease in odor sensitivity, while a positive score represents an increase in odor sensitivity in the sated when compared to the fasted state. Hence, odor sensitivity for chocolate decreases in metabolically impaired individuals with a high BMI after a meal, while no change in odor sensitivity is observed in metabolically healthy participants. However, we show a positive direct effect of BMI on change in odor sensitivity for chocolate depending on hunger state independent of metabolic health parameters. Thus, the effect of BMI on the change in odor sensitivity in response to a meal is positive after removing the effect of metabolic health (= adjusting for the mediators). This means that a high BMI is associated with an increase in odor sensitivity in the sated state, but only in metabolically healthy individuals. Further applied post hoc analyses underpinned this finding. We could show that participants with obesity outperformed those of normal weight while controlling for IR, but this effect was not present in the same design without IR as a covariate (original Hypothesis 2). Thus, individuals with obesity, who are metabolically healthy might have higher sensitivity for food odors in the sated state, while people affected by high HOMA-IR values independent of body weight status show lower olfactory sensitivity for food odors. Since we find these effects in the food odor condition only, our results support the proposed idea from Riera and colleagues [56], that reduced olfactory input protects against intake of high caloric diet by mimicking reduced odor sensitivity after meal intake. This might also reflect a specific reaction of our sensing system to food odor cues in response to our hunger state. In contrast to previous findings [51,52], we could not confirm any association between ghrelin and odor sensitivity in our data set. However, we found a weakly negative correlation between total ghrelin and BMI as has been previously reported [34].

Limitations

Although many important potential confounders such as menstrual cycle phase in women (see Table S1) are well controlled in the current study, there are limitations to consider as well. First, the hormonal profile in the current study is limited and may expand in future studies, assessing new hormonal and inflammatory markers that are possible targets to explain the relationship between obesity/metabolic health and olfactory perception. Secondly, the reliability in olfactory testing is generally rather low, possibly because of the susceptibility of our olfactory sense to many external factors such as smoking [57] and menstrual cycle [43,44]. While we attempted to counteract those influences by controlling for several confounding factors (smoking, season, cycle phase, and hunger), we were unable to address all possible confounders satisfactorily. Especially, since our measures rely on repeated sensitivity measurements, conclusions must be drawn with caution, because test–retest reliability ranges only from 0.43–0.86 in ODT testing [58–61]. However, we tried to counteract this weakness by randomizing the test day order. In addition, an unchanged sensitivity for chocolate could be due to the dissimilarities between the ODT test odor (chocolate) and the standard meal (cereal smoothie), as the greatest change would be expected when sensory specific satiety occurs [62]. Moreover, we did not include a direct measure of eating behavior. Hence, we cannot make a statement about the effect of odor sensitivity on food intake in everyday life.

5. Conclusions

This study supports recent literature on a close relationship between the olfactory system and metabolic parameters in obesity [51,52]. To our knowledge, it is the first study that shows a strong mediating effect of the metabolic health parameter insulin resistance on the relationship between BMI and odor sensitivity for chocolate. Thus, it provides further understanding of the pathophysiological mechanisms that might underlie altered smell perception in obesity.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/12/8/2201/s1, Figure S1: Sanity Check: Subjective hunger depicted with visual analogue ratings; Figure S2: Sanity Check: Physiological hunger depicted by pre- and postprandial insulin and ghrelin levels; Table S1: Olfactory sensitivity and sex/cycle phase in women; Table S2: Hormonal parameters in weight groups and correlation with BMI; Table S3: Mediation analysis. Relationship between BMI and odor sensitivity via hormonal parameters; and Table S4: Overview: Olfactory sensitivity between participants with normal weight and obesity for age and BMI.

Author Contributions: Conceptualization, M.P. and A.H.; methodology, M.P., K.W., and A.H.; software, M.P.; validation, M.P.; formal analysis, M.P.; investigation, M.P.; resources, A.H. and A.V.; data curation, M.P.; writing—original draft preparation, M.P.; writing—review and editing, J.F., K.W., and A.H.; visualization, M.P.; supervision, A.H. and A.V.; project administration, M.P. and A.H.; and funding acquisition, A.H. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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Study 3: Brain anatomical correlates of smell perception in obesity

Reduced olfactory bulb volume in obesity and its relation to metabolic health status

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Reduced Olfactory Bulb Volume in Obesity and Its Relation to Metabolic Health Status

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Smell perception plays an important role in eating behavior and might be involved in body weight gain. Since a body of literature implies that olfactory perception and function is hampered in obesity, we here investigate neuroanatomical correlates of this phenomenon. We assessed olfactory bulb (OB) volume with magnetic resonance imaging in 67 healthy participants with a body mass index (BMI) from 18.9 to 45.4 kg/m^2 (mean = 28.58 ± 6.64). Moreover, we obtained psychophysiological data on olfactory ability (Sniffin' Sticks, Food associated odor test) and self-report measurements on eating behavior. Additionally, we collected parameters associated with metabolic health in obesity (waist-hip ratio, waist-height ratio, leptin levels, body fat percentage, fat mass index, insulin resistance) to investigate recently proposed mechanistic explanatory models of why olfaction may be altered in obesity. We showed that OB volume was significantly lower in participants with obesity when compared to those of normal weight. Moreover, we found weak to moderate negative correlations between OB volume and BMI and related measures of metabolic health, especially leptin, body fat percentage, waist-height ratio and insulin resistance. However, neither OB volume nor BMI were related to olfactory function in our young and healthy sample. Nevertheless, our results provide first indications that obesity is associated with brain anatomical changes in the OBs.

Keywords: olfactory bulb, obesity, olfaction, smell perception, HOMA-IR, metabolic health

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INTRODUCTION

Obesity has become a worldwide epidemic with an increased risk for major individual health consequences and a severe burden to the healthcare system (Stevens et al., 2012). Since obesity is a risk factor for many diseases such as diabetes type 2, cardiovascular diseases, certain forms of cancer and stroke (Rosenthal et al., 2017), it is essential to understand the factors that accompany

excessive accumulation of body weight and its maintenance. One major factor driving the rapid increase of obesity is our obesogenic environment that encourages overconsumption of energy-dense foods even without physiological needs (Berthoud, 2012; Lopez-Gonzalez et al., 2020). Especially external cues, such as the smell of foods, can trigger appetite and intensify food cravings (Firmin et al., 2016). Thus, the olfactory system came into focus as an important contributor to unintentional weight gain.

In recent years, it has been shown that smell perception plays a significant role in the enjoyment of food and the control of food intake (Ramaekers et al., 2014, 2016; Proserpio et al., 2017). More specifically, olfaction influences food selection and meal size (Gaillet-Torrent et al., 2014) and triggers cephalic phase responses and cravings (Kitamura et al., 2010; Firmin et al., 2016; Proserpio et al., 2017). Hence, changes in olfactory perception might be involved in unhealthy eating and potentially lead to weight gain. On this notion, it has been shown that individuals with obesity show several alterations in the olfactory system. First, they have a higher hedonic response to palatable food odors when compared to people of normal weight (Stafford and Whittle, 2015). Second, it is widely accepted that individuals with obesity have decreased olfactory function (Richardson et al., 2004; Skrandies and Zschieschang, 2015; Fernández-Aranda et al., 2016; Fernandez-Garcia et al., 2017; Peng et al., 2019). Especially odor sensitivity, which reflects perceptual function (Hedner et al., 2010), is decreased in individuals with obesity when compared to people with normal weight (Skrandies and Zschieschang, 2015; Fernandez-Garcia et al., 2017).

The mechanisms behind these alterations remain unclear, however, it has been suggested that decreased olfactory function may be caused by hormonal and metabolic changes that are associated with obesity (Peng et al., 2019). This is supported by a recent finding of our working group, where we could show that high insulin resistance has a negative effect on food odor sensitivity in obesity (Poessel et al., 2020). Moreover, these hormonal changes might lead to altered function of the olfactory bulbs (Lacroix et al., 2015). Notably, the OBs have a high density of insulin and leptin receptors (Havrankova et al., 1981; Baskin et al., 1983; Marks et al., 1990; Thanarajah et al., 2019), hormones that are elevated in obesity and involved in homeostatic signaling (Murphy and Bloom, 2006; Durham, 2016; Lean and Malkova, 2016) as well as in modulating odor sensitivity (Tong et al., 2011; Brunner et al., 2013).

Interestingly, not all individuals with obesity show significant alterations in their metabolic and endocrine systems, referring to the concept of metabolically "healthy" obesity (Blüher, 2020). Those individuals are characterized by preserved insulin sensitivity and beta cell function, better cardiorespiratory fitness and lower visceral fat. In this respect, we believe that the olfactory system of metabolically healthy individuals with obesity might be less affected by these alterations. Consequently, we not only focus on body mass index (BMI) criteria for weight status, but additionally examine waist-height ratio (WHtR), waist-hip ratio (WHR), and leptin as proxies of body fat (Münzberg and Heymsfield, 2015; Christen et al., 2018; Landecho et al., 2019) and insulin resistance as assessed by homeostatic model assessment (HOMA-IR) (Gutch et al., 2015) and total body fat percentage as well as fat mass index (FMI).

The olfactory performance in standard tests (identification, discrimination and threshold tests) is reflected in the size of the olfactory bulb (OB) in participants with and without olfactory dysfunction (Buschhüter et al., 2008; Mazal et al., 2016). OB volume is reduced in various diseases that are associated with low olfactory performance, such as Parkinson's disease, Alzheimer's disease, schizophrenia, and depression (Turetsky et al., 2000; Thomann et al., 2009; Negoias et al., 2010; Liu et al., 2017). However, olfactory function was in most, but not in all cases negatively related to OB volume (Turetsky et al., 2000; Schriever et al., 2013). It is discussed that an insufficient afferent input of olfactory information from the olfactory receptor neurons to the OB causes the reduction in bulb volume (Gudziol et al., 2009). In addition, animal studies have reliably demonstrated that obesity leads to structural and functional changes in the olfactory system (Fadool et al., 2011; Thiebaud et al., 2014; Rivière et al., 2016). Since brain anatomical correlations to altered smell perception in human obesity have not been studied yet, we here investigate whether OB volume is associated with BMI and associated metabolic and hormonal markers of obesity, such as WHR, WHtR, body fat percentage, FMI, plasma level of leptin and insulin resistance. Further, we assessed olfactory function with the Sniffin' Sticks test battery. Moreover, we explore whether eating behavior as assessed by subjective reporting via questionnaires correlates with brain anatomical changes in the olfactory primary pathways.

MATERIALS AND METHODS

Participants

The sample consisted of 67 participants (33 women, 34 men), 28 participants had normal weight (BMI = 18.5 − 24.9 kg/m^2), 28 participants were obese (BMI > 30 kg/m^2), and 11 participants were overweight (BMI = 25 − 29.9 kg/m^2) (see **Table 1** for details). All participants were recruited from the Max Planck Institute for Human Cognitive and Brain Sciences database in Leipzig, Germany. They were aged between 21 and 41 years (28.5 ± 4.6 years) to minimize the impact of age on olfactory performance (Hummel et al., 2007). We excluded current or recent smokers (<3 years of abstinence) and subjects with allergies, history of nose surgery (except childhood adenoidectomy) and metabolic diseases (e.g., thyroid diseases or diabetes mellitus). Further exclusion criteria were vegetarian/vegan diet, history of neurological or psychiatric disorders, current use of medication (except oral contraceptives), drug use within the last 4 weeks and alcoholism. Pregnant and currently breastfeeding women were excluded for ethical reasons and because smell perception is altered in these conditions. All participants were previously screened by means of telephone interviews and had to meet our inclusion criteria (age between 18 and 45 years). After inclusion, participants provided written informed consent. The study was carried out in accordance with the Declaration of Helsinki and was approved by the Ethics

TABLE 1 | Sample characteristics.

	Total	Normal weight	Obese		Overweight
	Mean ± SD (range)			*p*-value/*F*-value between NW and OBE	
Characteristics					
n	67	28	28		11
Sex	33 ♀, 34 ♂	14 ♀, 14 ♂	14 ♀, 14 ♂		5 ♀, 6 ♂
Age (years)	28.5 ± 4.6 (21 – 41)	27.1 ± 4.3 (21 – 35)	29.5 ± 5.1 (21 – 41)	0.109/2.3[a]	29.5 ± 3.0
BMI (kg/m^2)	28.4 ± 6.6 (18.9 – 45.4)	22.3 ± 1.6 (18.9 – 24.9)	35.1 ± 4.3 (30.1 – 45.4)	<0.001***/212.834[a]	26.9 ± 1.4
WHR	0.89 ± 0.08 (0.75 – 1.05)	0.84 ± 0.06 (0.75 – 0.98)	0.93 ± 0.08 (0.80 – 1.05)	<0.001***/23.875[b]	0.88 ± 0.06
WHtR	0.52 ± 0.09 (0.38 – 0.71)	0.43 ± 0.04 (0.38 – 0.53)	0.61 ± 0.05 (0.51 – 0.71)	<0.001***/222.22[b]	0.51 ± 0.03 (0.45 – 0.55)
Body fat percentage (%)	28.43 ± 11.60 (8.21 – 60.60)	20.56 ± 6.02 (8.21 – 30.51)	37.50 ± 10.65 (11.99 – 60.60)	<0.001***/53.658[b]	25.39 ± 8.31
FMI	0.15 ± 0.09 (0.03 – 0.43)	0.08 ± 0.02 (0.03 – 0.11)	0.23 ± 0.08 (0.07 – 0.43)	<0.001***/92.03[b]	0.12 ± 0.50 (0.07 – 0.18)
BDI sum score	3.0 ± 3.3 (0 – 13)	2.0 ± 2.8 (0 – 11)	4.2 ± 3.8 (0 – 13)	0.018*/5.905[a]	2.5 ± 2.5
Passive smoking hours	1.5 ± 2.7 (0 – 15)	1.5 ± 2.9 (0 – 15)	1.5 ± 3.0 (0 – 15)	0.964/0.002[a]	1.5 ± 1.5
Hormonal profile					
HOMA-IR	1.14 ± 0.91 (0.10 – 5.10)	0.69 ± 0.38 (0.10 – 1.80)	1.73 ± 1.10 (0.60 – 5.10)	<0.001***/21.903[a]	0.78 ± 0.37
Leptin (ng/ml)	15.41 ± 13.91 (0.10 – 61.90)	8.13 ± 6.23 (0.10 – 27.70)	24.04 ± 15.95 (3.40 – 61.90)	<0.001***/21.878[b]	9.97 ± 8.04
Insulin (pmol/l)	60.18 ± 49.36 (7.00 – 278.30)	36.36 ± 19.82 (7.00 – 92.60)	91.68 ± 60.37 (30.40 – 278.30)	<0.001***/21.222[a]	40.59 ± 19.74
Glucose (mmol/l)	5.32 ± 0.43 (4.58 – 6.45)	5.15 ± 0.45 (4.58 – 6.16)	5.54 ± 0.39 (4.62 – 6.45)	0.001**/11.777[a]	5.18 ± 0.18

*Data are presented as mean values, standard deviations, and minimum and maximum values. [a]One-way analysis of variance (ANOVA). [b]One-way analysis of variance with the covariate sex (ANCOVA). BMI, body mass index; WHR, waist-hip ratio; WHtR, waist-height ratio; FMI, fat mass index; BDI sum score, sum score of Beck's Depression inventory; HOMA-IR, homeostatic model assessment of insulin resistance. *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.*

Committee of the University of Leipzig (Reference number 387/17-ek, date of vote: 2017-10-17).

Study Design

The data presented here are part of a larger, multi-centered study in Saxony, Germany. We here only present the data of participants that were tested in one location at the Max Planck Institute for Human Cognitive and Brain Sciences in Leipzig. Participants were tested on 2 consecutive days. On the first test day, we collected a blood sample after an overnight fasting period of approximately 12 h. All participants were screened for normal olfactory function with the short form of the Sniffin' Sticks odor identification test (Mueller and Renner, 2006). They further underwent a medical examination to assess body weight, height, waist and hip circumference and body fat percentage (measured by body impedance analysis). We conducted several questionnaires and interviews to assess eating behavior, past and present smoking behavior, including passive smoking, as well as information about the menstrual cycle phase of female participants. Moreover, we conducted a computer-based behavioral task and saliva tests on the first test day that are not part of the present data presentation in this manuscript. On the second test day, participants underwent after a 2 h fasting period a 50-min MRI scan, consisting of an anatomical and a functional part. After scanning, participants completed another set of questionnaires. In this manuscript, we only focus on the anatomical scan, the fMRI experiment is part of another subproject.

Materials

Olfactory Tests

Olfactory testing was performed with the commercially available Sniffin' Sticks® test battery (Sniffin' Sticks; Burghart Instruments, Wedel, Germany). It is a well-validated instrument to assess olfactory performance in the clinical and research context (Hummel et al., 1997). It includes subtests for odor threshold, odor discrimination and odor identification. In the threshold test, 16 triplets of felt-tip like pens are presented to the participant (Kobal et al., 1996; Hummel et al., 1997). In each triplet, one pen contains n-butanol, diluted in aqua conservans and concentrated from 1.22 ppm in pen number 16 up to 4% in pen number 1, whereas the other two pens contain only the solvent and serve

as blanks. The odorized pen of each triplet must be identified correctly, starting with the lowest concentration. Participants are blindfolded to avoid visual identification. In a single staircase procedure, a higher concentration is presented following an incorrect choice, and a lower concentration following two correct identifications in a row. This procedure is repeated until seven staircase reversals are reached. The threshold is defined as the mean of the four last reversals of the staircase. A higher score signals higher capacity to pick up odors from the environment. The odor discrimination test assesses the participant's ability to discriminate between different odorants. The task is to identify from three pens which one smells different than the other two pens. The odor identification test assesses the participant's ability to correctly identify commonly known odors from a list of four descriptors for each odorized pen. The odors are similar in intensity and include: orange, peppermint, turpentine, cloves, leather, banana, garlic, rose, fish, lemon, coffee, anise, cinnamon, liquorice, apple, pineapple. In each test the sum of correct answers can range from 0 to 16. The sum of all three subtests results in the composite TDI-score and reflects general olfactory capacity, it can range from 0 to 48. Hereby, a TDI score of $\geq$30.5 is defined as normosmia (=normal smell function), a score between 16.5 and 30 hyposmia (=reduced smell function) and $\leq$16.5 anosmia (=functional smell impairment) (Hummel et al., 2007). Additionally, we obtained odor identification data from a newly developed food associated odor test (FAOT), here the participants had to identify the correct odor from a list of four descriptors. The test was developed to examine how naturalistic food odors are perceived. It includes the following odors: cinnamon, vanilla, coconut, bell pepper, caraway, peppermint, marzipan, butter, peach, liquorice, grape juice, cacao, cooked beef, bread, chicken, and fish (Denzer-Lippmann et al., 2017). Questionnaires and Interviews.

We initially screened for inclusion and exclusion criteria and education, using a self-developed screening questionnaire assessing smoking and drinking behavior, health status and subjective olfactory function. Depressive symptoms were assessed with the Beck Depression Inventory (BDI) (Beck et al., 1961) in a paper pencil form at the beginning of the first test day to control for acute suicidal tendencies and exclude participants with a sum-score >18 since depression is a confounding factor for smell impairment (Negoias et al., 2010). Additionally, participants were face to face interviewed about their smoking status by means of a smoking interview that was previously implemented in the Leipzig LIFE study (Loeffler et al., 2015). The interview contains questions about their past and present smoking behavior as well as passive smoking. Eating behavior was assessed by means of the German version (Nagl et al., 2016) of the Three factor Eating Questionnaire (TFEQ) (Stunkard and Messick, 1985). The TFEQ describes three dimensions of human eating behavior: cognitive restraint of eating, disinhibition and hunger. Dietary Fat and Free Sugar Short Questionnaire (DFS) (Fromm and Horstmann, 2019) that obtains data about monthly intake of saturated fat and free sugar within the last year. Moreover, we used the German versions of the Food Craving Questionnaires for food (FCQ) (Meule et al., 2014) and chocolate (FCQ-C) (Meule and Hormes, 2015). Both scales obtain information about experienced food cravings. The FCQ collects information about general state and trait cravings for food while the FCQ-C assesses craving and hunger for chocolate specifically. Furthermore, we obtained information on the menstrual cycle of women to assess cycle phase, since odor sensitivity is higher in follicular phase of the cycle / under oral contraception and lower in the luteal phase (Derntl et al., 2013; McNeil et al., 2013).

Blood Collection

Venous blood samples were collected after a fasting period of approximately 12 h, using 5.5 ml Sarstedt S-Monovette containers prepared with clotting activator for serum leptin and insulin and 2.7 ml Fluoride/EDTA preparations for glucose. Glucose tubes were immediately centrifuged at 3500 r.p.m. at 10°C and serum after standing at room temperature for 30 min. Both, serum and glucose were separated in two 1 ml tubes (Eppendorf Safe-Lock Tubes) and immediately after centrifugation frozen at -80°C.

Neuroimaging

Brain imaging data were acquired using a 3 Tesla Siemens SKYRA scanner equipped with a 20-channel head coil. An MPRAGE (Mugler and Brookeman, 1990) dataset was acquired to create a T1-weighted (T1w) image. *TR* = 2,300 ms, echo time; *TE* = 2.98 ms, inversion times; *TI* = 900 ms (non-selective inversion recovery), flip angle; *FA* = 9°, nominal resolution = 1 mm isotropic. Right and left OB volume were determined using multislice T2-weighted turbo spin-echo images, with *TR*/*TE*/*FA* = 6,630 ms/126 ms/160°, acquired spatial resolution = 0.5 × 0.5 (in-plane), 1 mm slice thickness, 30 slices (no slice gap), and signal averages = 2.

Data Analysis

R version 3.4.3. within RStudio (RStudio Team, 2016) was used for statistical evaluation. We used BMI as a continuous variable or as a grouping variable (normal weight: BMI 18.4 − 24.99 kg/m^2, obese: BMI > 30 kg/m^2). We used WHR, WHtR, circulating leptin levels, body fat percentage and FMI as additional measures of weight status, that are more related to metabolic health. Especially, WHtR and FMI have been identified as reliable predictors of metabolic risk in obesity (Łopatyński et al., 2003; Liu et al., 2013). Homeostasis Model Assessment of insulin resistance (HOMA-IR score) (Matthews et al., 1985), an index that serves as a proxy of insulin resistance, was then calculated from insulin and glucose by means of the HOMA2 Calculator[1], applying the formula: HOMA-IR = glucose [mmol/l] × insulin [pmol/l]/135.

The α-level was set at 0.05. Bonferroni correction was applied to adjust the α-level for multiple testing. Whenever statistical assumptions for parametric testing were violated, we applied non-parametric robust tests. We defined outliers as values below or above 2.2 interquartile range from the samples lower or upper quartile (Hoaglin and Iglewicz, 1987).

Olfactory bulb size was assessed with 3D Slicer software (Fedorov et al., 2012), version 4.10.2[2]. We employed the

[1]https://www.dtu.ox.ac.uk/homacalculator/index.php
[2]https://www.slicer.org/

planimetric contouring method (Rombaux et al., 2009). The examiner manually delineates the OBs in the coronal slices and multiplies each surface area (mm^2) by slice thickness (1 mm). The posterior end of the OBs is reached when encountering two equal sized, narrower surface areas in a row. Finally, all obtained volumes are added up for the total volume of each bulb. OB measurements were performed by three independent experimenters who were blinded for the weight status and sex of the participants. The mean of their results serves as the OB volume.

First, we applied a group design to display differences between BMI groups as defined by international standards on OB volume while controlling for sex. Assumption tests showed homogeneity of variances/covariances, as assessed by Box's M test. There were no univariate/multivariate outliers, as assessed by standardized residuals greater than ±3 standard deviations/Mahalanobis distance. Second, the relationship between OB volume and measures that reflect body weight status (BMI, WHR, body fat percentage, leptin) was assessed by correlation analysis. We performed partial Spearman's rank correlation for not normally distributed variables (BMI, WHR, leptin, insulin, HOMA-IR, age, TDI score, odor identification, odor discrimination, craving questionnaires, eating behavior questionnaires, olfaction questionnaires) with OB volume. Since OB volume is smaller in women when compared to men, we used sex as a covariate. Additionally, we used partial Pearson's product moment correlation to depict the relation between normally distributed variables (body fat percentage, odor threshold, DFS data). We interpreted the strength of the correlation according to Cohen's conventions (Cohen, 1988).

We further used SPSS (version 22.0, SPSS Inc., Chicago, IL) for the replication of a mediation analysis to disentangle the effect of insulin resistance on the relationship between obesity and olfactory function. Unstandardized indirect effects were computed for each of 10,000 bootstrapped samples, and the 95% confidence interval was computed by determining the indirect effects at the 2.5th and 97.5th percentiles.

RESULTS

Sample Characteristics

Participant characteristics are given in **Table 1**. Descriptive values for the total sample as well as for BMI subgroups are listed. **Table 1** contains general characteristics (age, depressive symptoms, information about passive smoking), metabolic health parameters (BMI, WHR, body fat percentage) and hormonal parameters (plasma insulin, glucose and leptin, HOMA-IR score). BDI scores indicated no or only mild depressive symptoms in all subjects (mean = 3.01, *SD* = 3.35, range: 0–13), however, participants with obesity had significantly more depressive symptoms than participants with normal weight. As expected participants significantly differed in BMI, WHR, and body fat percentage. Participants with normal weight had also significantly lower HOMA-IR scores as well as lower levels of plasma insulin, glucose and leptin when compared to obese participants.

Psychophysical Function

Olfactory function assessed by Sniffin' Sticks and food associated odor test (FAOT) are given in **Table 2**. A one-way multivariate analysis of variance was run to determine the effect of weight status (normal weight vs. obese) on olfactory performance (Odor identification, odor discrimination, odor identification, TDI sum score) while controlling for sex. There was no statistically significant difference between weight groups in all olfactory tests, $F(3, 51) = 0.389$, $p = 0.761$ (**Table 2**).

In addition, none of the olfactory tests was associated with OB volume or BMI as determined by Pearson's product moment correlation and Spearman's rank-order correlation (**Table 2**).

Relationship Between OB Volume and BMI/Other Measures That Are Associated With Metabolic Health (WHR, WHtR, Body Fat Percentage, FMI, Leptin, Insulin Resistance)

Means, range and standard deviations of OB volume and whole brain volume grouped by weight status and in total are depicted in **Table 3**. Firstly, we explored group differences in OB volume between participants with obesity and normal weight, we found a significant group effect as determined by one-way ANCOVA [$F(1, 52) = 8.119$, $p = 0.004$] with smaller volume in individuals with obesity (**Figure 1**). Additionally, we checked whole brain volume to control the specificity of this effect. There was no difference in whole brain volume in the weight groups [$F(1, 52) = 1,691$, $p = 0.682$].

Additionally, we investigated the relationship between OB volume and BMI as a continuous variable as well as other measures of metabolic health in obesity using correlation analysis. A partial Spearman's rank-order correlation was run to determine the relationship between BMI and OB volume whilst controlling for sex. There was a weak, negative correlation between OB volume and BMI, which was statistically significant ($r = -0.278$, $n = 65$, $p = 0.028$) (**Figure 2**). Further, we assessed other measures that are associated with metabolic health status in obesity: HOMA-IR, leptin, body fat percentage, WHtR, WHR, and FMI (**Figure 2**). A partial Pearson's product moment correlation was run to determine the relationship between body fat percentage and OB volume. There was a weak, negative correlation between OB volume and body fat percentage, which was statistically significant ($r = -0.273$, $n = 65$, $p = 0.031$). A partial Spearman's rank-order correlation was run to determine the relationship between OB volume and HOMA-IR/leptin/WHtR/WHR/FMI whilst controlling for sex. There were statistically significant weak to moderate, negative correlations for HOMA-IR and OB volume ($r = -0.258$, $n = 65$, $p = 0.041$), leptin and OB volume ($r = -0.253$, $n = 65$, $p = 0.045$) as well as for WHtR and OB volume ($r = -0.321$, $n = 65$, $p = 0.010$). However, there were no statistically significant correlations between OB volume and WHR ($r = -0.199$, $n = 65$, $p = 0.118$) or FMI ($r = -0.216$, $n = 65$, $p = 0.089$) whilst controlling for sex.

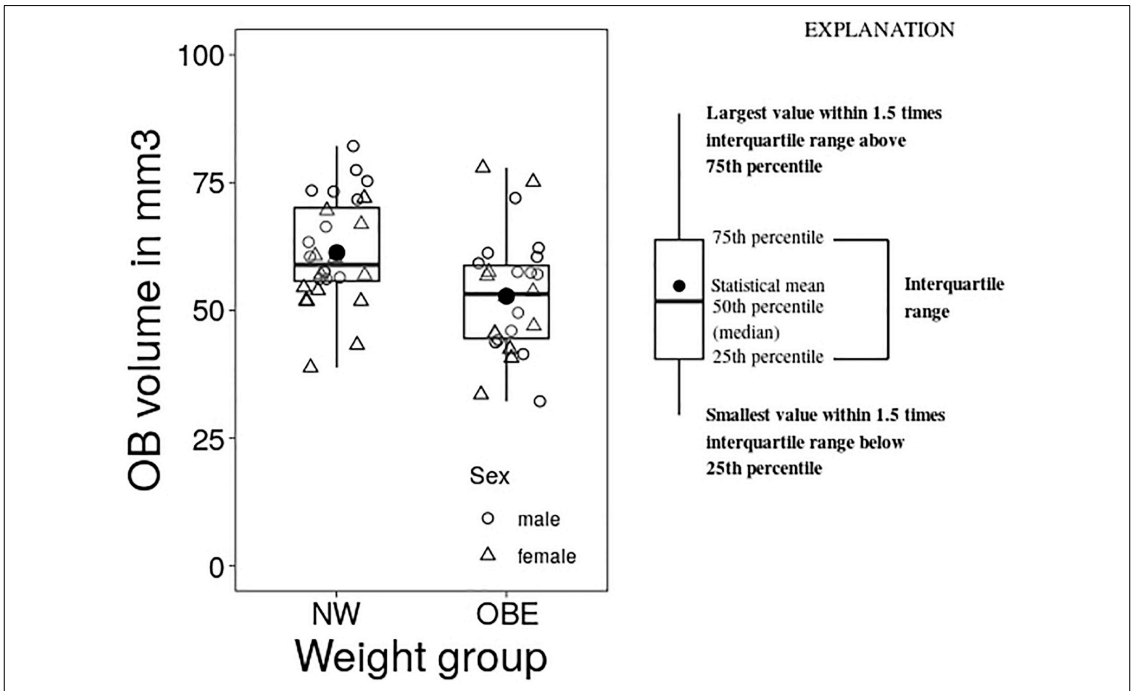

FIGURE 1 | Group differences between normal weight (NW) and obese (OBE) participants in OB volume as assessed by one-way analysis of variance with the covariate sex (ANCOVA).

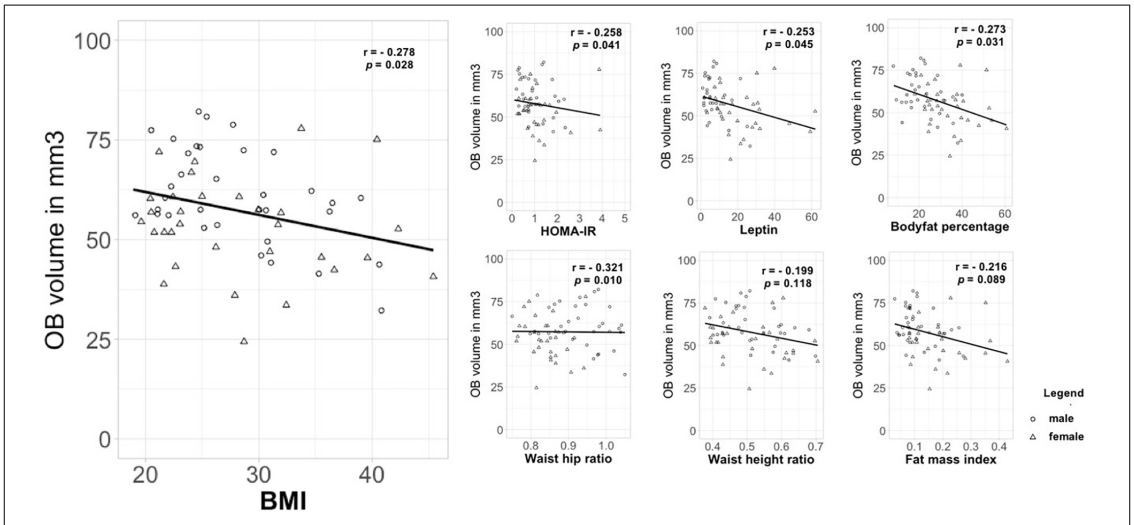

FIGURE 2 | Correlation between OB volume and measures that are associated with metabolic health status in obesity. OB volume in mm^3; BMI in kg/m^2; HOMA-IR, homeostatic model assessment of insulin resistance; leptin in ng/ml; body fat in percentage; WHtR, waist-height ratio; WHR, waist-hip ratio in cm; FMI, fat mass index.

TABLE 2 | Olfactory performance assessed with the Sniffin' Sticks battery and FAOT.

	Total (n = 67)	Correlation with BMI	Correlation with OB volume	Normal weight (n = 28)	Obese (n = 28)	p-value/F-value between NW and OB	Overweight (n = 11)
	Mean ± SD (range)	p-value/r		Mean ± SD (range)			
Smell tests							
Threshold	7.63 ± 1.85 (1.75 – 11.00)	0.393/ – 0.109[a]	0.348/0.120[a]	7.65 ± 2.19 (1.75 – 11.00)	7.56 ± 1.61 (4.25 – 10.50)	0.959/0.030[b]	7.75 ± 1.65
Discrimination	12.45 ± 2.12 (6 – 16)	0.938/0.010[a]	0.505/0.086[a]	12.11 ± 2.67 (6 – 16)	12.64 ± 1.66 (10 – 16)	0.863/0.812[b]	12.82 ± 1.54
Identification	13.55 ± 1.51 (9 – 16)	0.268/-0.142[a]	0.807/0.003[a]	13.54 ± 1.17 (11 – 15)	13.43 ± 1.91 (9 – 16)	0.801/0.064[b]	13.91 ± 1.14
TDI sumscore	33.63 ± 3.90 (22.75 – 40.25)	0.230/-0.133[a]	0.244/0.149[a]	33.29 ± 4.60 (22.75 – 40.25)	33.63 ± 3.54 (26.25 – 38.50)	0.758/0.096[b]	34.48 ± 2.85
FAOT	13.4 ± 1.47 (9 – 16)	0.272/-0.141[a]	0.854/0.024[a]	13.29 ± 1.27 (10 – 15)	13.39 ± 1.50 (11 – 16)	0.774/0.083[b]	13.73 ± 1.90

Data are presented as mean values, standard deviations, and minimum and maximum values. [a]Spearman's rank-order correlation. [b]One-way analysis of variance with the covariate sex (ANCOVA). BMI, body mass index; TDI sumscore, sumscore of odor threshold, odor discrimination, and odor identification; FAOT, food associated odor test.

TABLE 3 | Olfactory bulb (OB) and whole brain volume (WBV) overall participants and for weight groups.

	Total	Normal weight (n = 28)	Obese (n = 26)	p-value/F-value between NW and OBE	Overweight
	Mean ± SD (range)				
OB volume right	56.95 ± 12.83 (26.21 – 82.47)	61.01 ± 10.67 (26.21 – 82.47)	52.05 ± 10.94 (30.59 – 74.87)	**0.002**/9.272[a]**	58.19 ± 18.33
OB volume left	57.59 ± 13.35 (22.59 – 84.25)	61.58 ± 11.25 (22.59 – 82.74)	53.51 ± 13.07 (32.44 – 80.90)	**0.016*/5.959[a]**	57.07 ± 16.83
Mean OB volume	57.27 ± 12.64 (24.40 – 82.13)	61.29 ± 10.33 (24.40 – 80.83)	52.78 ± 11.64 (32.30 – 77.89)	**0.004**/8.119[a]**	57.63 ± 17.27
WBV	1.20 ± 0.10 (0.96 – 1.39)	1.21 ± 0.10 (1.04 – 1.33)	1.20 ± 0.10 (1.00 – 1.39)	0.682/.169[a]	1.18 ± 0.08

*[a]One-way analysis of variance with the covariate sex (ANCOVA). OB volume in mm^3, WBV in liter; NW, normal weight; OBE, obese. *$p \leq 0.05$; **$p \leq 0.01$; Bold font indicates statistical significance.*

Replication: Mediation Effect of HOMA-IR on the Relation Between BMI and Smell Function

The relationship between BMI and olfactory function, as assessed by TDI score, was mediated by HOMA-IR. The standardized regression coefficient was significant between BMI and the mediator HOMA-IR (r = 0.625, p = 0.001) and between TDI score and the mediator HOMA-IR (r = 0.348, p = 0.042). The standardized indirect effect between BMI and TDI score via HOMA-IR was -0.154 (CI -0.268 and -0.036). There was no significant direct effect for BMI and TDI score (r = 0.126, p = 0.164, CI: -0.053 and 0.304).

Relation Between Eating Behavior and BMI/OB Volume

A Spearman' rank-order correlation was applied to investigate the relationship between BMI and eating behavior questionnaires. We found a weak, positive correlation between BMI and cognitive restraint as well as hunger scale of the TFEQ (r = 0.258, n = 61, p = 0.041; r = 0.249, n = 61, p = 0.049).

A partial Spearman's rank-order correlation was run to determine the relationship between eating behavior questionnaire data and OB volume whilst controlling for sex. There was a weak, positive correlation between OB volume and chocolate craving (sum score from the FCQ-Trait Chocolate questionnaire), which was statistically significant (r = 0.278, n = 59, p = 0.033). All correlational data of BMI and OB volume with eating behavior questionnaires are given in **Supplementary Table 1**.

Yet we treat these findings with caution, since they would not survive alpha level correction for multiple testing.

DISCUSSION

In this study, we demonstrate for the first time that OB volume is reduced in individuals with obesity when compared to those with normal weight. Additionally, we found weak to moderate negative correlations between OB volume and BMI as well as

other measures of metabolic health in obesity, such as body fat percentage, WHtR, leptin levels and insulin resistance. Our results imply that, similar to other diseases such as depression and Parkinson's disease (Negoias et al., 2010; Li et al., 2016), obesity also involves a neuroanatomical change in the OBs compared to healthy participants with normal weight.

Since we could show that whole brain volume was not associated with BMI and weight status in our sample, we conclude that our observation is not related to a general atrophy in our obese sample but might be specific to the olfactory system. However, since we have not measured the volume of other sensory regions, such as the gustatory, visual or auditory cortex, we cannot conclude with certainty that our results are specific to this one sensory system or might also affect other sensory areas. Additionally, as our study groups did not differ in age, we can exclude a frequently observed effect of age on the sense of smell in obesity due to older obese study populations when compared to those of normal weight, as discussed in a recent review paper by Peng and colleagues (Peng et al., 2019).

Yet, what might be the underlying causes for smaller OB volume in obesity? Our observation poses the question whether OB volume reduction is involved in the development of obesity or a consequence of this condition. First, it might be possible that altered olfactory processing leads to changes in eating behavior and consequently favors weight gain. Second, it might be possible that reduction in OB volume is a consequence of obesity and is for instance caused by metabolic and endocrine impairments. Both questions cannot be fully addressed by the design of our study. Nonetheless, we assume that the latter point of view is more likely, since it has been shown that olfactory dysfunction is more pronounced in severe obesity when compared to overweight or moderate obesity (Richardson et al., 2004; Pastor et al., 2016; Fernandez-Garcia et al., 2017).

Surprisingly, we could not find a relationship between OB volume or BMI and psychophysiological measures of olfactory ability, respectively. This is striking for two reasons: (1) obesity is commonly associated with low olfactory ability and (2) we expected that lower OB volume in obesity is associated with lower olfactory function. As to the first point, we think that our very young and healthy obese sample may be the reason for the preserved olfactory ability. As discussed by the authors of this paper it is plausible that individuals with obesity that are metabolically healthy may have normal olfactory function (Poessel et al., 2020). This point of view is in line with another finding that especially people that are morbidly obese when compared to moderately obese are affected from limitations in olfactory function (Richardson et al., 2004). In this regard, proposed explanatory models of impaired olfactory function in obesity might explain this phenomenon: They point to an important role of metabolic and hormonal changes in obesity that may cause altered smell perception (Fernandez-Garcia et al., 2017; Peng et al., 2019). For instance, leptin, which is often increased in obesity while their brain is insensitive to this hormone, has an inhibitory role on olfactory function in mice (Getchell et al., 2006). In line with this reasoning, it is interesting that we could replicate our results from Poessel et al. (2020) in this sample. We find a strong mediating effect of insulin resistance as assessed by HOMA-IR score on the relationship between BMI and olfactory function as assessed by TDI score. This implies that high BMI is associated with lower olfactory function via IR. As to the second point, one can speculate that reduced OB volume in obesity is an early sign of the pathophysiological changes in olfactory function. Therefore, obesity and associated metabolic changes might have negative effects on the olfactory system before hampering obvious psychophysical function. Especially, young age, short duration of obesity and being metabolically healthy might protect against decline of olfactory function (Attems et al., 2015; Lacroix et al., 2015; Riera and Dillin, 2016). Yet, it is unexpected that the alteration of smell perception does not first manifest itself in behavior, but in the brain anatomy. Typically, a bottom-up mechanism is discussed as the cause of decreasing OB volume, for instance in chronic rhinosinusitis (Han et al., 2017) and smoking (Schriever et al., 2013). In those conditions, lower afferent input is thought to reduce OB volume because of inflammatory processes in the nose or due to toxic effect of smoke-associated products on the olfactory epithelium. This bottom-up mechanism of olfactory dysfunction has been shown in animal models: deprivation of olfactory function leads to decreased OB volume (Cummings et al., 1997). Interestingly, this effect is reversible after restoring olfactory function. In line with that result is an observation in humans, where surgical treatment of patients with chronic rhinosinusitis results in increasing OB volume (Gudziol et al., 2009). However, since we detect a low OB volume without obvious olfactory dysfunction in our obese sample, it might be possible that function is preserved due to redundant information processing: the specific anatomy and physiology of the olfactory system with its parallel processing pathways could ensure that olfactory function is maintained. Olfactory sensory neurons project to homologous glomeruli on the level of the olfactory bulb, in a way that odor information is represented and processed in two mirror maps (Nagao et al., 2002; Sato et al., 2020). From there, the olfactory information is transferred to higher order olfactory brain regions in a parallel manner (Savic et al., 2000; Nagayama et al., 2010; Payton et al., 2012). With regard to our data, we assume that based on the parallel circuitry of odor information processing at the level of the olfactory bulb, a reduction in the volume of OBs does not necessarily lead to behavioral deficits.

Secondly, a top-down process explanation seems also probable, as for instance discussed in Parkinson's Disease (Li et al., 2016) and depression (Negoias et al., 2010). Here, neurodegenerative processes or disrupted salience processing in the brain with a bias toward internal thoughts disregarding the exteroceptive sensory system may lead to a reduction of OB volume (Rottstädt et al., 2018). Especially, the loss of sensory neurons in obesity (O'Brien et al., 2017) might result in a decrease of sensory function. This has been shown in various sensory systems, such as the auditory (Hwang et al., 2013) and olfactory system. More specifically, Thiebaud et al. (2014) reported that hyperlipidemic diet in mice and associated obesity leads to a loss of olfactory sensory neurons and a decrease in olfactory function (Thiebaud et al., 2014). Moreover, it might be possible that the neural response to sensory input is blunted as it has been shown in taste processing (Weiss et al., 2019).

It has recently been shown that hormones whose homeostatic signaling may be impaired in obesity (Baly et al., 2007; Aime et al., 2012; Russo et al., 2018), modulate olfactory performance in humans. More specifically, intranasally applied insulin as well as intravenously applied ghrelin improve olfactory function (Tong et al., 2011; Thanarajah et al., 2019). Interestingly, the OBs have a high density of receptors for these hormones. Thus, the OBs might be directly affected by these alterations. In this respect, we think that another approach to explain diminished OB volume in the absence of olfactory dysfunction might be plausible: metabolic and hormonal dysfunction in obesity might directly affect the neurogenesis and synaptogenesis of the OB. Interestingly, chronic inflammation, as observed in obesity, is associated with the disruption of hippocampal neurogenesis (Chesnokova et al., 2016) and lower hippocampal and gray matter volumes (Tsai et al., 2019). Especially, body fat percentage, leptin and insulin resistance are among other indicators of increased fat mass and might thus be associated with chronic low-grade inflammation in obesity (Jung and Choi, 2014; Chen et al., 2015; Saad et al., 2016; La Cava, 2017; Reilly and Saltiel, 2017). Interestingly, it has been found in rats that diet induced type 2 diabetes leads to a decreased electrophysiological response of olfactory neurons (Rivière et al., 2016). Moreover, obesity and chronically high levels of insulin disrupt the metabolic sensing of the OBs in mice (Fadool et al., 2011). In the light of these findings, one can speculate that the negative correlation between OB volume and markers of metabolic health might be a hint that neurogenesis of the OBs might be affected by obesity associated inflammatory processes.

Another, albeit highly speculative, possibility could be that smells might be differently processed in individuals with obesity. Following the observation of Weiss et al. (2020) that people without apparent OBs can still smell, one can speculate that other structures in the brain might take over the processing of olfactory information by the formation of a glomerular space somewhere else in the cortex. In this respect, we assume that although the OB volume might be lower in our obese sample, the olfactory ability might be preserved by other structures.

Since the OBs are structures that respond directly to odors and play an important role in the processing and dissemination of olfactory information to higher order brain regions (Rombaux et al., 2009), they may also play a crucial role in homeostatic signaling and eating behavior. Thus, we examined the relationship between OB volume and eating behavior as assessed by subjective questionnaires on cravings (FCQ), actual food intake (DFS) and eating style (TFEQ). We found weak positive correlations between OB volume and chocolate craving whilst controlling for sex and BMI. This means that the higher chocolate craving the bigger are the OBs. These results should be interpreted prudently since this correlation would not survive alpha level correction for multiple testing. However, we think this is a first interesting link between brain anatomy in the olfactory system and eating behavior.

Although we have planned our study carefully, there are limitations to consider. First, in the context of eating behavior and obesity we would encourage researchers to develop or use further instruments to assess olfactory perception. Albeit the standard olfactory Sniffin' Sticks test battery is a well-validated measure of olfactory function, it might not be as sensitive to depict subtle differences in healthy subjects, because it was primarily developed for the evaluation of olfactory loss. Moreover, the investigation of eating behavior can be extended in future studies. Especially, we would suggest that direct and implicit measurements of eating behavior should be carried out to avoid bias through subjective reporting. In addition, we consider it necessary to determine the onset and duration of obesity in future studies in order to make a more reliable statement about the influence of obesity associated changes in the metabolic and endocrine systems on olfactory function. In this context, we think an extension of the hormonal profile and collecting inflammatory markers would be compelling. Additionally, we think that our results only provide first indications that the olfactory system is neuroanatomically altered in people with obesity. Further studies should also look at other structures of the primary and secondary olfactory pathways to understand whether reduction in OB volume also affects higher regions of olfactory processing.

In sum, our study finds solid evidence that OB volume is decreased in obesity while sensory function appears to be preserved. Furthermore, we show negative correlations between OB volume and insulin resistance, leptin levels and body fat. This might provide a mechanistic link between changes in the olfactory system in obesity.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the University of Leipzig (Reference number 387/17-ek, date of vote: 2017–10–17). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MP, TH, and AH: conceptualization. MP, NB, and AH: methodology. MP and NB: software, investigation, and writing—original draft preparation. MP: validation, data curation, and visualization. MP, AJ, and NB: formal analysis. AH and AV: resources. MP, AH, TH, NB, and AV: writing—review and editing. AH, TH, and AV: supervision. MP and AH: project administration and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnhum.2020.586998/full#supplementary-material

Supplementary Table 1 | Correlation analysis of eating behavior questionnaires and BMI/OB volume.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

IV. SUMMARY

Dissertation zur Erlangung des akademischen Grades Dr. rer. nat.

The association between metabolic health status and smell perception in obesity: behavioral and brain anatomical correlates

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Obesity is a major health concern that is accompanied by a high risk for several disorders such as type 2 diabetes, cardiovascular disease and certain forms of cancer (Stevens et al., 2012; Lahey and Khan, 2018). Since the prevalence of obesity has nearly tripled within the last 45 years and is still on the rise (WHO, 2018), it is imperative to understand mechanisms that might underlie the emergence and maintenance of obesity to develop new prevention- and intervention strategies. The high availability of energy-rich food is one of the main contributing factors for the increasing prevalence of obesity. Hence, the mechanisms underlying eating without physiological needs come into focus. In that regard, the olfactory system plays a major role: it is equally involved in homeostatic signaling of hunger and hedonic eating (Palouzier-Paulignan et al., 2012). Given that the sense of smell is altered in obesity (for an overview see Peng et al., 2019), the overall goal of this thesis is to contribute to further understanding of the mechanisms that might underlie this phenomenon.

It has been previously shown that people with obesity evaluate food odors as more pleasant (Stafford and Whittle, 2015) and show higher reactivity towards them (Proserpio et al., 2019), however, they persistently have lower olfactory function. This low function is most evident in

olfactory sensitivity, i.e. picking up odors from the environment, and is therefore related to appetite and food search. Odor sensitivity evaluation is usually a lengthy procedure and is standardly performed with non-food odors (n-butanol or phenyl ethyl alcohol). However, Stafford and Whittle (2015), revealed a different result for sensitivity to food odors: obese outperformed normal-weight participants for chocolate odor. Strikingly, further scrutiny reveals that metabolic and endocrine health factors could provide a possible explanation for divergent results of olfactory sensitivity to food and non-food odors in obesity: whereas the chocolate-study included only class 1 obese participants (BMI 30-35 kg/m^2), all other studies included class 2-3 obese participants (BMI > 35 kg/m^2). It is likely that obese class 2-3 participants are more affected by hormonal changes, such as higher insulin resistance and higher leptin levels than their less obese counterparts. On a recent note, it has been shown that the olfactory and endocrine systems are closely linked (Palouzier-Paulignan et al., 2012). As such there are many receptors for hunger-related hormones located in brain structures that are highly relevant for odor processing as well as for the regulation of homeostatic needs (Baly et al., 2007; Lacroix et al., 2008; Henkin, 2010). Especially, the olfactory bulbs, where olfactory information is firstly processed in the brain, have a high density of insulin and leptin receptors (Baskin et al., 1983; Thanarajah et al., 2019; Havrankova et al., 1981; Marks et al., 1990). Further, animal studies have reliably demonstrated that obesity leads to structural and functional changes in the olfactory system (Thiebaud et al., 2014; Fadool et al.2011; Riviére et al., 2016). However, brain anatomical changes in the olfactory system of humans have not been studied yet.

To conclude, we firstly aimed to develop an olfactory test that is easy to administer and of short duration to apply in a complex research design, because available tests are time consuming and highly variable in duration (10-25 min). Secondly, in order to elucidate the potential link between olfactory impairments in obesity and metabolic health factors, we investigated food and non-food odor sensitivity in a wide body weight range and related it to metabolic and endocrine factors such as insulin resistance. Third, we aimed to investigate the possible relationship between obesity and brain anatomical changes in the olfactory bulbs.

Study 1: In our first study, we measured olfactory sensitivity in a within-subject repeated-measures design in 20 young and healthy participants. Using the odor detection threshold subtest from the "Sniffin' Sticks" test battery, we applied three different presentation methods: (1) gold standard, (2) shorter single staircase method and (3) ascending procedure. Compared to the gold-standard, the shorter single staircase procedure was 26% and the ascending

procedure was 51% shorter in duration. Both short procedure thresholds correlated highly with the gold standard threshold. All three tests showed similar test-retest reliability. To conclude, we have developed a test that takes on average 5-7 minutes less time and is as reliable as the gold standard.

Study 2: Within the second study, we focused on metabolic health parameters that might explain the relationship between odor sensitivity and obesity. We investigated food and non-food odor sensitivity in the hungry and sated state in 75 young healthy participants with normal weight, overweight and obesity in a within-subject, repeated-measures design. We assessed metabolic health status with BMI, WHR, pre- and postprandial levels of insulin, leptin, glucose, and ghrelin. We showed that odor sensitivity did not directly depend on body weight status or BMI. However, we found a strong negative mediating effect of insulin resistance as assessed by HOMA-IR score on the relationship between BMI and olfactory sensitivity for the food odor. Post-hoc regression models revealed that insulin resistance rather than obesity is responsible for this effect. To conclude, our findings indicate a strong negative association between insulin resistance and sensitivity to food odors.

Study 3: In the third study, we examined neuroanatomical correlates of smell perception in obesity and its relationship with metabolic health factors. Olfactory bulb volume was assessed with magnetic resonance imaging in 67 healthy normal weight, overweight and obese participants. To examine recently proposed mechanistic explanatory models of altered smell perception in obesity, we collected parameters that are associated with metabolic health in obesity, such as insulin resistance, leptin, body fat percentage and fat mass index. We showed that in our sample, people with obesity had significantly lower olfactory bulb volume when compared to people with normal weight. Further, we found that olfactory bulb volume was negatively associated with other measures of metabolic health, especially insulin resistance, leptin, and body fat percentage. Our results imply that, similar to other diseases such as depression and Parkinson's disease, obesity also involves a neuroanatomical change in the olfactory bulbs compared to healthy participants with normal weight. Hence, our study provides first indications that obesity is associated with brain anatomical changes in the olfactory bulbs.

Study Limitations

Although the studies have been carefully designed and planned, there are some limitations to consider. Firstly, albeit the test-retest reliability in the first study is comparable to that typically found for olfactory testing, the coefficients indicate a moderate, or even poor reliability (Koo and Li, 2016). This is most likely due to a generally rather low test-retest reliability in olfactory testing, because the olfactory sense is very susceptible to various factors such as smoking (Hayes and Jinks, 2012), menstrual cycle (Derntl et al., 2013; McNeil et al., 2013), climate (Katotomichelakis et al., 2007) and attention (Negoias et al., 2010). This makes it very difficult to examine the sense of smell. While we attempted to counteract several confounding factors (smoking, season, cycle phase), we were unable to address all possible confounders satisfactorily, in particular hunger state in study 1. While we advised participants to refrain from eating two hours prior testing to avoid being either hungry or sated, we did not control food intake or quantify hunger state. In the second study, we were again confronted with the problem of low test-retest reliability. We counteract this weakness by randomizing the test day order and provided fasted participants with a standard meal. Nonetheless, both studies must be interpreted with caution due to general low test-retest-reliability in olfactory assessments. Moreover, the hormonal profile in study 2 and 3 is very limited. It may expand in future studies to obtain more information about new hormonal and inflammatory markers that are possible targets to explain the relationship between metabolic health and olfactory perception. Further, the investigation of eating behavior could be expanded in future studies and may include direct measurements of eating behavior to avoid bias due to subjective reporting in questionnaires. Therefore, we cannot make a statement about the effect of odor sensitivity on food intake in everyday life. In addition, the results from study 3 deliver only first indications of neuroanatomical alterations in the olfactory system of individuals with obesity. Future studies should additionally investigate structures of the primary and secondary olfactory pathways to understand whether neuroanatomical changes in olfactory bulbs influence olfactory processing in higher order brain regions.

Conclusion

The overall aim of this thesis was to shed light on the complex relationship between obesity and olfaction. Study 1 provides two easy-to-use odor threshold test procedures for clinical use or for complex research designs with limited time frames. Importantly, this thesis emphasizes the major role of metabolic health status and especially insulin resistance in the altered smell perception in obesity. Most notably, poor metabolic health mediates the relationship between

obesity and olfactory sensitivity (study 2). Metabolic health parameters rather than obesity per se might be responsible for low olfactory function and should be further scrutinized in future studies. In particular, a group-design with elevated vs. normal HOMA-IR participants instead of BMI groups could provide more insights. Intriguingly, a high BMI and related metabolic health factors, such as high insulin resistance and high body fat percentage are associated with neuroanatomical changes in the olfactory system, i.e., lower olfactory bulb volume (study 3). These findings contribute to a further understanding of explanatory models introduced by Peng et al. (2019). In accordance with this metabolic and hormonal model our results support the theoretical framework that metabolic and hormonal shifts in obesity might be crucial for changes in olfactory perception. Thereby, these results provide a deeper understanding of the pathophysiological mechanisms underlying altered olfactory function in obesity. Subsequently, olfaction might represent a new target for prevention or therapy.

Articles included in this thesis:

Poessel, M., Breuer, N.[†], Joshi, A., Pampel, A., Villringer, A., Hummel, T. & Horstmann, A. (2020). Reduced olfactory bulb volume in obesity and its relation to metabolic health status. *Frontiers in Human Neuroscience*.

Poessel, M., Freiherr, J., Wiencke, K., Villringer, A. & Horstmann, A. (2020). Insulin resistance is associated with reduced food odor sensitivity across a wide range of body weights. *Nutrients* 2020, *12*, 2201.

Poessel, M., Freiherr, J. & Horstmann, A. (2019). Rapid assessment of olfactory sensitivity using the „Sniffin Sticks“. *Chemosensory Perception*, 1-8.

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VI. APPENDIX

A. DECLARATION OF AUTHENTICITY

Erklärung über die eigenständige Abfassung der Arbeit

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne unzulässige Hilfe oder Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Ich versichere, dass Dritte von mir weder unmittelbar noch mittelbar eine Vergütung oder geldwerte Leistungen für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen, und dass die vorgelegte Arbeit weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zweck einer Promotion oder eines anderen Prüfungsverfahrens vorgelegt wurde. Alles aus anderen Quellen und von anderen Personen übernommene Material, das in der Arbeit verwendet wurde oder auf das direkt Bezug genommen wird, wurde als solches kenntlich gemacht. Insbesondere wurden alle Personen genannt, die direkt an der Entstehung der vorliegenden Arbeit beteiligt waren. Die aktuellen gesetzlichen Vorgaben in Bezug auf die Zulassung der klinischen Studien, die Bestimmungen des Tierschutzgesetzes, die Bestimmungen des Gentechnikgesetzes und die allgemeinen Datenschutzbestimmungen wurden eingehalten. Ich versichere, dass ich die Regelungen der Satzung der Universität Leipzig zur Sicherung guter wissenschaftlicher Praxis kenne und eingehalten habe.

....

Datum Unterschrift

B. Author Contributions

Spezifizierung des eigenen wissenschaftlichen Beitrags

Im Rahmen der vorliegenden Promotion wurden drei Originalarbeiten in peer-reviewed Fachzeitschriften veröffentlicht. Die Spezifizierung des eigenen wissenschaftlichen Beitrags ist tabellarisch für jede Studie einzeln aufgelistet.

1. **Beiträge der Autoren – Studie 1:** Poessel, M., Freiherr, J. & Horstmann, A. (2018). Rapid assessment of olfactory sensitivity using the „Sniffin Sticks". *Chemosensory Perception*, 1-8. Doi:10.1007/s12078-019-09261-z.

	Poessel, M.	Freiherr, J.	Horstmann, A.
Studiendesign	x	x	x
Datenerhebung	x		
Datenanalyse	x		x
Manuskriptentwurf	x		
Manuskript-Überarbeitung	x	x	x

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Beiträge der Autoren – Studie 2: Poessel, M., Freiherr, J., Wiencke, K., Villringer, A. & Horstmann, A. (2020). Insulin resistance is associated with reduced food odor sensitivity across a wide range of body weights. *Nutrients,* 12, 2201; doi:10.3390/nu12082201

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Studiendesign	x				x
Datenerhebung	x				
Datenanalyse	x		x		x
Manuskriptentwurf	x				
Manuskript-Überarbeitung	x	x	x	x	x

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Beiträge der Autoren – Studie 3: Poessel, M.†, Breuer, N.†, Joshi, A., Pampel, A., Villringer, A. & Hummel, T., Horstmann, A. (2020). Reduced olfactory bulb volume in obesity and its relation to metabolic health status. *Frontiers in Human Neuroscience*, doi: 10.3389/fnhum.2020.587763.

† Diese Autoren haben gleichermaßen zu dieser Arbeit beigetragen und teilen sich die Erstautorenschaft.

	Poessel, M.	Breuer, N.	Joshi, A.	Pampel, A.	Villringer, A.	Hummel, T.	Horstmann, A.
Studiendesign	x					x	x
Methodik der Studie	x	x		x			x
Datenerhebung	x	x					
Validierung der Daten, Datenaufbereitung & Visualisierung der Daten	x						
Formale Datenanalyse	x	x	x				
Dateninterpretation	x	x					
Manuskriptentwurf	x	x					
Manuskript-Überarbeitung	x	x	x	x	x	x	x

ERKLÄRUNG ZU GETEILTER ERSTAUTORENSCHAFT

Die Erstautorenschaft für Studie 3 ist zwischen mir und Frau Nora Breuer geteilt. Beide Erstautorinnen hatten hierbei einen eigenständigen wissenschaftlichen Beitrag, der jeweils selbstständig bearbeitet wurde (siehe Übersicht der Autorenbeiträge). Die Publikation zu Studie 3 ist sowohl Teil der kumulativen Promotion zum Dr. rer. nat. von Frau Pössel als auch zum Dr. med. von Frau Breuer.

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